UNIVERSITY OF ALBERTA

FACTORS INFLUENCING FERTILITY, PREINCUBATION EMBRYO DEVELOPMENT, AND EMBRYO VIABILITY IN DOMESTIC FOWL

BY

GAYLENE MARIE FASENKO

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled FACTORS INFLUENCING FERTILITY, PREINCUBATION EMBRYO DEVELOPMENT, AND EMBRYO VIABILITY IN DOMESTIC FOWL submitted by GAYLENE MARIE FASENKO in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL REPRODUCTION.

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DATE: Feb 23 '92

DEDICATION

To my parents, grandparents, and brother

from whom I have gained more valuable knowledge

than I could ever obtain at university.

ABSTRACT

The overall objective of this thesis was to identify specific factors in domestic fowl which influence fertility, hatchability, embryo viability, and development of the embryo prior to incubation. In the first study, the effects of varying the nest holding period and the method of egg storage on embryo development were evaluated. Embryo development, in all four experiments in this thesis, was evaluated using a modified Eyal-Giladi and Kochav classification. The experiment was a 2 x 2 factorial design with two nest holding times (0 to 1 h and 6 to 7 h) and two egg storage methods (eggs packed or unpacked). Nest holding time affected embryonic development, but storage method, and nest holding time x storage method interaction did not. Embryo development was more advanced in eggs which were held for long versus short periods in the nest.

Two nest holding durations under conditions of high environmental temperatures were tested to determine the influence of these factors on fertility, and the relationship between embryo growth and viability at the time of storage. Eggs were collected and stored within 1.5 h of oviposition, or after 6 to 7 h in the barn. Embryos of eggs which remained in the nest for longer periods were more developed than embryos of eggs collected shortly after laying, however, fertility, hatchability, viability, and the incidence of early dead embryos (died between 0 to 7 d of incubation) were not affected by the duration of the nest holding period. These results indicate that embryo viability is not related to the stage of preincubation embryo development at the time of storage.

Eggs were stored for different lengths of time to determine the

effects on embryo development and mortality. Within 1 h of oviposition the eggs were collected, weighed, and assigned to one of five storage treatment groups of 0, 4, 7, 14, or 21 d. Duration of storage, and fresh egg weight did not affect embryonic development. A linear relationship was observed between duration of storage and loss of egg weight during storage. Embryo mortality was related to loss of egg weight. Loss of egg weight and embryo mortality were highest in eggs which were stored for a longer period of time. This study provides evidence that lengthening the duration of the storage period does not increase embryo growth, but does increase embryonic mortality.

The final objective was to investigate if hen age and egg sequence position were related to fertility, hatchability, viability (hatch of fertile eggs), and preincubation embryo development in broiler breeders. All eggs laid during the period of 31-54 wk of age were collected daily and the sequence position (first or subsequent) of each egg determined. Fertility and hatchability were not related to egg sequence position, but were related to hen age. Older hens demonstrated lower fertility and hatchability. In contrast, embryo viability and preincubation embryo development, were not related to hen age, but were related to egg sequence position. First of sequence eggs had lower viability, and embryos of these eggs were more developed than embryos of subsequent eggs. These results indicate that the reduction in chick production observed as the hen ages may be due to the increased incidence of first of sequence eggs.

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LIST OF ABBREVIATIONS

C - celcius

CP - crude protein

d - day(s)

g - gram(s)

h - hour(s)

kcal - kilocalories

m - meter(s)

ME/kg - metabolizable energy per kilogram

mm - millimeter(s)

SD - standard deviation

wk - week(s)

I. GENERAL INTRODUCTION

The reproductive system of *Galliform* birds is unique. Unlike mammals, chickens have only one functioning ovary. Regression of the right ovary begins at 10 d of incubation (Johnson, 1986), such that by the time the hen reaches reproductive maturity, only the left ovary and oviduct are functioning.

The ovary of the mature hen consists of follicles arranged in a hierarchy. There are usually seven to ten large yellow follicles (10 to 35 mm in diameter) evident on the ovary (Etches *et al.*, 1983). These follicles are designated as F1 to F10, with the F1 follicle being the largest, most mature follicle which is the next to ovulate. Once this follicle has ovulated the next largest follicle will mature and eventually ovulate. Also present are five to ten small yellow follicles (5 to 10 mm in diameter), ten large white follicles (2 to 3 mm) and thousands of small white follicles (1 mm or less) (Robinson and Etches, 1986). These small follicles are recruited into the large follicle hierarchy when they reach approximately 10 mm in diameter (Etches, 1990). Many small follicles become atretic before reaching this size. However, once the follicles have been recruited into the small yellow follicle hierarchy and begin depositing yellow yolk, it is very rare that they become atretic (Etches *et al.*, 1984).

The oviduct of the hen is comprised of five regions: infundibulum, magnum, isthmus, shell gland (uterus), and the vagina. Upon ovulation, the follicle is dropped into the body cavity and subsequently retrieved by the infundibulum (North, 1990), where it stays for approximately 15 to

30 minutes (Warren and Scott, 1935). If the infundibulum fails to retrieve the follicle, the ovum remains in the body cavity. This is referred to as internal ovulation. Normally, during the time the follicle is in the infundibulum, the follicle is fertilized if sperm are present. Deposition of the four layers of albumen occurs in the magnum (North, 1990) where the follicle resides for 2 to 3 h (Warren and Scott, 1935). Rotation of the egg as it travels down the oviduct produces two twisted cords of albumen referred to as chalazae, which serve to centralize the yolk inside the egg (North, 1990). During the 1 to 2 h passage of the follicle through the isthmus (Warren and Scott, 1935), inner and outer shell membranes are formed (Johnson, 1986). It is in the shell gland where the developing egg spends most of its time, taking from 20 to 26 h to pass through (Warren and Scott, 1935). Before deposition of the shell, the developing egg takes up water and electrolytes through the shell membrane in a process called "plumping" (North, 1990). During a period of approximately 15 h, calcification of the shell is completed, with shell pigmentation deposited in the last 5 h (Warren and Conrad, 1942). At the junction of the uterus and the vagina are sperm storage organs called utero-vaginal sperm host glands (UV-SHG). It is because of these glands that mating usually need only occur once per week for fertile eggs to be produced. Sperm present in these glands are sequentially replaced by sperm from the most recent insemination (Compton *et al.*, 1978). Sperm storage glands are also present in the infundibulum. The main function of the vagina is to aid in oviposition of the egg. Normally, prior to oviposition the egg rotates, thus allowing oviposition to occur large end first (North, 1990). Transport of the egg throughout the oviduct is

mainly accomplished by muscular contractions (Johnson, 1986).

Chickens usually lay one egg per day with days of consecutive egg laying called a clutch or a sequence. The word clutch is most often used when describing the laying pattern of determinate species that lay eggs once per year, while the term sequence is used when describing species, such as turkeys and chickens, which lay an indeterminate number of eggs in repetition throughout the year. Sequences are separated by days of non-laying called "pause" days. The production of an egg, from ovulation to oviposition, usually takes from 24 to 28 h (Etches, 1990). With the exception of the first egg of a sequence, ovulation occurs approximately .5 to 1 h after oviposition, which means that as a sequence progresses, the eggs are laid later each day (Etches, 1990). The variation in time between oviposition of successive eggs in a sequence is a reflection of the length of a sequence; eggs within long sequences take a shorter period of time to be formed than eggs within short sequences (Johnson, 1986). The first egg of a sequence is usually oviposited early in the day. These follicles, although mature, remain on the ovary for approximately 16 h longer than follicles of subsequent eggs of a sequence, and take 40 to 44 h to be laid from the time of the previous oviposition, due to the pause day (Robinson *et al.*, 1991). Multiple ovulation in chickens may occur if two follicles mature and are ovulated at the same time, or if one premature follicle is ovulated along with a mature follicle (Zelenka, *et al.*, 1986).

Follicular maturation and ovulation in birds depends upon environmental and physiological factors. Regulation of the light and dark period (photoperiod) during each day "sets" a circadian rhythm or

"biological clock" in the hen (Etches, 1990). The most commonly used photoperiod for mature hens is 14 light:10 dark (North, 1990). It is the change from light to dark which provides the cue for the circadian rhythm, which entrains the timing of oviposition referred to as the "open period" (Etches, 1990). The open period is defined as the approximate 8 h period of the day that a pre-ovulatory surge of luteinizing hormone (LH) can occur (Etches, 1990). Plasma concentrations of this gonadotrophin peak 4 to 6 h before ovulation (Etches *et al.*, 1981), and are responsible for initiating ovulation. The duration of the open period restricts oviposition to a 6 to 8 h period of the day (Etches, 1990). Therefore, when a follicle reaches maturity outside the timing of the open period, a pause day results, and the follicle must wait until the beginning of the next open period to be ovulated. The physiological mechanisms controlling the open period have not been defined.

Prior to reaching 10 mm in diameter, ovarian follicles produce dehydroepiandrostendione, androstendione, and estrogens in response to LH stimulation *in vitro* (Robinson and Etches, 1986; Etches, 1990). Although the role of androgens in follicular maturation is unknown, estradiol is responsible for yolk deposition (Johnson, 1986). As follicles mature, they gradually lose the ability to produce these hormones and increase production of progesterone (Robinson and Etches, 1986). The F1 follicle produces copious amounts of progesterone (Etches and Cunningham, 1976) which stimulates the secretion of gonadotrophin releasing hormone (GnRH) produced in the hypothalamus (Etches, 1990). The release of GnRH into the hypothalamic/pituitary portal system prompts the release of a pre-ovulatory surge of LH from the anterior pituitary (Etches, 1990). The

functions of LH are to induce ovulation and to promote steroidogenesis (Robinson and Etches, 1986; Robinson *et al.*, 1988). In response to the LH surge, progesterone production in the largest follicle increases (Etches, *et al.*, 1981). Thus there is a positive feedback relationship between progesterone and LH (Etches and Cunningham, 1976; Etches *et al.*, 1983). GnRH also causes the release of follicle stimulating hormone (FSH) from the anterior pituitary. The importance of FSH in ovarian steroidogenesis in the chicken has yet to be determined.

Development of the avian embryo begins immediately after fertilization in the infundibulum, and continues within the body of the hen, at 41.5°C (Whittow, 1986), for about one day. After fertilization, the follicle travels the length of the oviduct to the shell gland. By this point a flat disk of active cytoplasm, called the germinal disc, has formed on the surface of the yolk and is beginning to cleave (Eyal-Giladi, 1984). For the next 0 to 11 h in the shell gland, cleavage of the germinal disc continues (Stages I to VI of Eyal-Giladi and Kochav, 1976). Briefly, cleavage involves the division of the cytoplasm into cells called blastomeres. During the cleavage process, vertical and horizontal cleavage furrows are evident in the center of the germinal disc, and continue to spread out from the center as cleavage progresses (Eyal-Giladi, 1976). The cleavage process continually diminishes the size of the blastomeres. By the end of cleavage, the germinal disc, now referred to as a blastodisc (Eyal-Giladi, 1984), is approximately five or six cells thick at the center and one to two cells thick on the periphery (Bellairs, 1971). The blastodisc is separated from the yolk by a fluid-filled cavity; the subgerminal cavity (Eyal-Giladi, 1984).

The final 8 to 9 h spent in the shell gland involves the first morphogenetic movements of blastodisc cells into two distinct regions; the area pellucida and the area opaca (Stages VII to X of Eyal-Giladi and Kochav, 1976). Shedding of the lower cells of the blastodisc initiates the formation of these two areas (Eyal-Giladi and Kochav, 1976). The cells are shed into the subgerminal cavity starting at the future posterior end of the blastodisc, and gradually moving toward the anterior end (Eyal-Giladi, 1984). These shed cells appear to disintegrate (Eyal-Giladi, 1984). This results in the transformation of a central portion of the five to six cell layer blastodisc, to a transparent, one cell layer section called the area pellucida. On the periphery of the circular area pellucida is a non-transparent or opaque ring, five to six cells thick known as the area opaca. As these two areas are differentiating, the diameter of the blastodisc is also increasing (Eyal-Giladi and Kochav, 1976). The blastodisc is now referred to as a blastoderm (Eyal-Giladi, 1984).

At the time of laying, about 60,000 cells are present in the chick blastoderm (Burley and Vadehra, 1989). Further development of the blastoderm requires incubation of the egg at temperatures of approximately 37 to 38°C. Upon incubating, the second morphogenetic event, hypoblast formation, is observed (Stages XI to XIV of Eyal-Giladi and Kochav, 1976). Observation of the lower or ventral side of the blastoderm, reveals a "horseshoe" shaped cluster of cells collecting at the posterior end of the blastoderm. This group of cells, known as Koller's sickle, marks the beginning of the formation of the hypoblast (Eyal-Giladi and Kochav, 1976). Migration of the hypoblast cells continues across the ventral

surface of the blastoderm until a solid circle of hypoblast cells is complete. At this point the blastoderm is arranged as follows: starting from the periphery of the blastoderm, there is a "ring" of area opaca, then a "transparent belt" of area pellucida, and in the center of the blastoderm is the solid circle of hypoblast cells. The final developmental stage prior to primitive streak formation involves the formation of a "cellular bridge", at the posterior end of the blastoderm, between the hypoblast and the area opaca (Eyal-Giladi and Kochav, 1976).

Primitive streak formation occurs due to morphogenetic movements in the epiblast which are induced by hypoblast cells (Bellairs, 1986). This event marks the beginning of gastrulation in the bird. Although early investigators did not specifically state what stage of development they referred to as gastrulation, it is likely that they viewed the onset of hypoblast formation as early gastrulation. This was due to the presumption that hypoblast cells eventually formed the endoderm layer (see Romanoff, 1960; and Rol'nik, 1970 for review). The primitive streak appears as a thickening at the posterior border of the area pellucida approximately 6 to 7 h into incubation (Stage 2 of Hamburger and Hamilton, 1951). By 12 to 13 h of incubation the long, narrow primitive streak has progressed to approximately half way across the area pellucida (Stage 3 of Hamburger and Hamilton 1951), and at 18 to 19 h the primitive streak is fully formed (Stage 4 of Hamburger and Hamilton, 1951). At this point the area pellucida is "pear" shaped (Hamburger and Hamilton, 1951). During the formation of the primitive streak, epiblast cells leave the ectoderm layer and ingress into the embryo through a structure in the center of the primitive streak known as the primitive groove. The

ingressing cells eventually form mesoderm and endoderm cell layers (Bellairs, 1986). Along with the ectoderm, these three cell layers eventually form all the structures in the chick.

After 19 to 22 h of incubation, the primitive streak regresses in an anterior to posterior fashion, in the opposite direction in which it formed (Stage 5 of Hamburger and Hamilton, 1951). It is replaced by a "rod of condensed mesoderm" known as the notochord (Hamburger and Hamilton, 1951). A fold in the blastoderm marks the anterior end of the embryo (Stage 6 of Hamburger and Hamilton, 1951). After this phase of development the most notable structural changes between 23 to 53 h of incubation are the appearance of pairs of somites. These structures, composed of dorsal mesoderm, are formed on either side of the notochord (Stages 7 to 14 of Hamburger and Hamilton, 1951.) Incubation of the egg to 21 d is required for development to continue and subsequent hatching to occur.

There are many factors which affect embryo development in the domestic fowl. Temperature is the primary catalyst which influences development. Embryonic diapause in chickens may be initiated after oviposition by exposing the egg to temperatures below physiological zero. Physiological zero is defined as the temperature below which embryonic development ceases. This temperature has been reported at two levels; 20 to 21°C (Edwards, 1902; Proudfoot and Hamilton, 1990) and 27°C (Funk and Biellier, 1944). The differences in physiological zero reported may be due to different methods of measuring embryo growth. It has been demonstrated that fertile eggs may be stored for short periods of time (approximately 1 wk), and then development initiated without harming the

viability of the embryo (Proudfoot and Hamilton, 1990).

Management practices such as frequency of egg collection and method of egg storage may also influence embryo development. Eggs which remain in the barn for extended periods of time may contain embryos which are developmentally more advanced, due to longer periods of incubation by the hen, or because of exposure to environmental temperatures above physiological zero. Heywang (1945) conducted one of the few experiments involving various collection frequencies, methods of storage, and exposure to different temperatures. From this study Heywang (1945) concluded that eggs do not have to be gathered more than once per day during hot days, but should be stored in a cool place to maintain viability (# eggs hatch/# fertile eggs). Storage methods of eggs may affect development by influencing the rate of cooling of the egg to below physiological zero. North (1984) determined that it takes about .75 d to cool eggs from 32.2 to 18.3°C when eggs are stored on incubator trays, and 1 to 2 d when eggs are stored in cases with holes in the sides. Embryo viability is reduced when eggs are stored (Byng and Nash, 1962; Mather and Laughlin, 1976, 1977; Whitehead *et al.*, 1985), and hatching is delayed (Mather and Laughlin, 1976). Pre-storage warming of eggs to be stored for more than 1 wk has been recommended to increase hatchability (# eggs hatch/# eggs set) (Butler, 1991). To maintain hatchability it is recommended that eggs stored for less than 1 wk are held at 16 to 17°C, and eggs stored for periods longer than 1 wk are stored at 11 to 12°C (Proudfoot and Hamilton, 1990).

In the absence of natural mating, frequency of insemination in chickens has been identified as a factor affecting fertility (# eggs

fertile/# eggs set), embryo development, and viablility. Fertility may be reduced due to a decrease in number of sperm in the UV-SHG (Pierson *et al.*, 1988) or an inability of older sperm to fertilize the ovum (Nalbandov and Card, 1943). A decrease in embryo development (Nalbandov and Card, 1943), and an increase in embryonic death (Lodge *et al.*, 1971) and abnormalities (Dharmarajan, 1950) has been demonstrated in embryos fertilized by aged sperm.

Many factors which may affect fertility, hatchability, viability, and preincubation embryo development are related to age of the hen. One factor is egg weight. As the hen ages, egg weight increases due to a decrease in ovulation rate (Bahr and Palmer, 1989). Al-Murrani (1978) observed that the weight of embryos, after 20 d of incubation, from large eggs were significantly higher than the weight of embryos from small eggs. However, no effect of egg weight on embryo weight was observed prior to 14 d of incubation. Position of an egg in a sequence is another possible factor. It has been demonstrated that as hens age, the incidence of first of sequence eggs increases due to a decline in sequence length (Robinson *et al.*, 1990). As previously stated, first eggs of a sequence eggs remain on the ovary for about 16 h longer than subsequent eggs, and are therefore subject to preovulatory aging. Research conducted on turkeys provided evidence that first of sequence eggs had reduced fertility, hatchability, and viability compared with subsequent eggs of a sequence (Bacon and Nestor, 1979). Robinson *et al.* (1991) determined that fertility and viability after 7 d of incubation were not affected by sequence position, but embryo production (fertility x viability) was significantly reduced in first versus subsequent eggs of a sequence. Taylor and Gunns (1935)

found that embryos of first of sequence eggs were larger in size than embryos of subsequent eggs.

The factors which influence stage of embryonic development may be important as there may be an optimum stage of embryonic development at which to store eggs to maintain viability. Nicolaides (1933) and Hays and Nicolaides (1934) observed that embryos from hens with high hatchability were developed to further stages of gastrulation than embryos from hens with low hatchability. However, Taylor and Gunns (1935) determined that the diameter of the area pellucida, and the amount of overgrowth were not correlated with hatchability of the hen.

The overall objective of this thesis was to further identify in domestic fowl, some of the factors which contribute to variability in stage of preincubation embryo development, fertility, hatchability, and viability. The specific objectives of the various research projects were to: 1) determine if the amount of time the egg spends in the nest, and the method of egg storage during cooling, affects preincubation embryo development (Chapter II);

2) establish if embryos of eggs remaining in the nest for short or long periods under conditions of high environmental temperatures, were at different stages of embryo development; and if there were differences in development, did this have an affect on embryo viability (Chapter III);

3) resolve if all embryonic development ceases during long term storage at temperatures below physiological zero (Chapter IV); and

4) identify the effects of hen age and egg sequence position on fertility, hatchability, viability, and preincubation embryo development (Chapter V).

LITERATURE CITED

Al-Murrani, W.K., 1978. Maternal effects on embryonic growth and post-embryonic growth in poultry. Br. Poult. Sci. 19:277-281.

Bacon, W.L., and K.E. Nestor, 1979. Reproductive traits of first eggs of clutches vs. other clutch positions in turkeys. Poultry Sci. 58:257-258.

Bahr, J.M., and S.S. Palmer, 1989. The influence of aging on ovarian function. Crit. Rev. Poult. Biol. 2:103-110.

Bellairs, R., 1971. Page 69 *in*: Developmental Processes in Higher Vertebrates. Logo Press Limited, London, England.

Bellairs, R., 1986. The primitive streak. Anat. Embryol. 174:1-14.

Burley, R.W., and D.V. Vadehra, 1989. The Avian Egg. Chemistry and Biology. Page 271. John Wiley and Sons, Inc. New York, NY.

Butler, D.E., 1991. Egg handling and storge at the farm and hatchery. Pages 195-203 *in*: Avian Incubation. Poultry Science Symposium Number Twenty-two. S.G. Tullet, ed. Butterworth-Heinemann, London, England.

Byng, A.J., and D. Nash, 1962. The effects of egg storage on hatchability. Br. Poult. Sci. 3-4:81-86.

Compton, M.M., H.P. Van Krey, and P.B. Siegel, 1978. The filling and emptying of uterovaginal sperm-host glands in the domestic hen. Poultry Sci. 57:1696-1700.

Dharmarajan, M., 1950. Effect on the embryo of staleness of the sperm at the time of fertilization in the domestic hen. Nature 165:398.

Edwards, C.L., 1902. The physiological zero and the index of development for the egg of the domestic fowl. Am. J. Physiol. 6:351-397.

Etches, R.J., 1990. The ovulatory cycle of the hen. Crit. Rev. Poult. Biol. 2:293-318.

Etches, R.J., F. Croze, and C.E. Duke, 1981. Plasma concentrations of luteinizing hormone, progesterone, testosterone, and estradiol in follicular and peripheral venous plasma during the ovulation cycle of the hen. Adv. Physiol. Sci. 33:89-98.

Etches, R.J., and F.J. Cunningham, 1976. The interrelationship between progesterone and luteinizing hormone during the ovulation cycle of the hen (*Gallus domesticus*) J. Endocrinol. 71:51-58.

Etches, R.J., H.E. MacGregor, T.F. Morris, and J.B. Williams, 1983. Follicular growth and maturation in the domestic hen (*Gallus domesticus*). J. Reprod. Fert. 67:351-358.

Etches, R.J., J.B. Williams, and J. Rzasa, 1984. Effect of corticosterone and dietary changes in the hen on ovarian function, plasma LH and steroids and the response to exogenous LHRH. J. Reprod. Fert. 70:121-130.

Eyal-Giladi, H., and S. Kochav, 1976. From cleavage to primitive streak formation: A complementary normal table and a new look at the first stages of the development of the chick. I. General Morphology. Dev. Biol. 49:321-337.

Eyal-Giladi, H., 1984. The gradual establishment of cell commitments during the early stages of chick development. Cell Diff. 14:245-255.

Funk, E.M., and H.V. Biellier, 1944. The minimum temperature for embryonic development in the domestic fowl (*Gallus domesticus*). Poultry Sci. 23:538-540.

Hamburger, V., and H.L. Hamilton, 1951. A series of normal stages in the development of the chick embryo. J. Morph. 88:49-92.

Hays, F.A., and C.N. Nicolaides, 1934. Variability in development of fresh laid hens eggs. Poultry Sci. 13:74-89.

Heywang, B.W., 1945. Gathering and storing hatching eggs during hot weather. Poultry Sci. 24:434-437.

Johnson, A.L., 1986. Reproduction in the Female. Pages 403-431 *in*: Avian Physiology, 4th ed. P.D. Sturkie, ed. Springer-Verlag, New York, NY.

Lodge, J.R., N.S. Fechheimer, and R.G. Jaap, 1971. The relationship of *in vivo* sperm storage interval to fertility and embryonic survival in the chicken. Biol. Reprod. 5:252-257.

Mather, C.M., and K.F. Laughlin, 1976. Storage of hatching eggs: The effect on total incubation period. Br. Poult. Sci. 17:471-479.

Mather, C.M., and K.F. Laughlin, 1977. Storage of hatching eggs: The effect on early embryonic development. Br. Poult. Sci. 18:597-603.

Nalbandov, A., and L.E. Card, 1943. Effect of stale sperm on fertility and hatchability of chicken eggs. Poultry Sci. 22:218-226.

Nicolaides, C.N., 1933. Relation of time of laying and embryonic mortality. Poultry Sci. 12:274-276.

North, M.O., 1984. Page 77 *in:* Commercial Chicken Production Manual, 3rd ed. AVI Publishing Company, Westport, CT.

North, M.O., 1990. Pages 36-41 *in*: Commercial Chicken Production Manual, 4th ed. AVI Publishing Company, New York, NY.

Pierson, E.E., G.R. McDaniel, and L.M. Krista, 1988. Relationship between fertility duration and *in vivo* sperm storage in broiler breeder hens. Brit. Poult. Sci. 29:199-203.

Proudfoot, F.G., and R.M.G. Hamilton, 1990. Care of Hatching Eggs Before Incubation. Publication 1573/E, Communications Branch, Agriculture Canada, Ottawa, ON, Canada.

Robinson, F.E., and R.J. Etches, 1986. Ovarian steroidogenesis during follicular maturation in the domestic fowl (*Gallus domesticus*). Biol. Reprod. 35:1096-1105.

Robinson, F.E., R.J. Etches, C.E. Anderson-Langmuir, W.H. Burke, K.W. Cheng, F.J. Cunningham, S. Ishii, P.J. Sharp, and R.T. Talbot, 1988. Steroidogenic relationships of gonadotropin hormones in the ovary of the hen, (*Gallus domesticus*). Gen. Comp. Endocrinol. 69:455-466.

Robinson, F.E., R.T. Hardin, and A.R. Robblee, 1990. Reproductive senescence in domestic fowl: egg production, sequence length, and inter-sequence pause length. Brit. Poult. Sci. 31:871-879.

Robinson, F.E., R.T. Hardin, N.A. Robinson, B.J. Williams, 1991. The influence of egg sequence position on fertility, embryo viability, and embryo weight in broiler breeders. Poultry Sci. 70:760-765.

Rol'nik, V.V., 1970. Pages 85-87 *in*: Bird Embryology. Israel Program for Scientific Translations, Ltd. Jerusalem, Israel.

Romanoff, A.L., 1960. Pages 160-165 *in*: The Avian Embryo. Structural and Functional Development. The Macmillan Company, New York, NY.

Taylor, L.W., and C.A. Gunns, 1935. Size of unincubated embryo in relation to hatchability. Poultry Sci. 14:294.

Warren, D.C., and R.M. Conrad, 1942. Time of pigment deposition in brown-shelled hen eggs and in turkey eggs. Poultry Sci. 21:515-520.

Warren, D.C. and H.M. Scott, 1935. The time factor in egg formation. Poultry Sci. 14:195-207.

Whitehead, C.C., M.H. Maxwell, R.A. Pearson, and K.M. Herron, 1985. Influence of egg storage on hatchability, embryonic development, and vitamin status in hatching broiler chicks. Br. Poult. Sci. 26:221-228.

Whittow, G.C., 1986. Regulation of body temperature. Pages 221-268 *in*: Avian Physiology, 4th ed. P.D. Sturkie, ed. Springer-Verlag, New York, NY.

Zelenka, D.J., P.B. Siegel, and H.P. Van Krey, 1986. Ovum formation and multiple ovulation in lines of White Plymouth Rocks and their crosses. Br. Poult. Sci. 27:409-414.

II. VARIABILITY IN PREINCUBATION EMBRYO DEVELOPMENT IN EGG-TYPE DOMESTIC FOWL. 1. EFFECTS OF NEST HOLDING TIME AND METHOD OF EGG STORAGE

INTRODUCTION

In the chicken there is considerable variability in the stage of embryonic development at oviposition (Tretyakov, *et al.*, 1973). After oviposition, environmental temperature plays a large role in the rate of embryonic development (Kaplan *et al.*, 1978). In embryos less than 18 d old it has been determined that the internal temperature of the egg stays very close to that of the environmental temperature (Romijn and Lokhorst, 1955). Romanoff (1939) determined that temperature influences on embryonic growth rate are greatest during early embryonic development. Management factors such as the frequency of egg collection will influence how long a freshly laid egg is exposed to environmental housing conditions. The method of egg storage during cooling may also affect the rate of egg cooling.

Much research in the past has focused on storage conditions that may affect embryo viability and hence hatchability of fertile eggs. Many studies have reported the effects of length of holding period (Byng and Nash, 1962; Reinhart and Hurnik, 1976) and holding conditions such as daily turning (Proudfoot, 1966), and temperature (Olsen and Haynes, 1947; Reinhart and Hurnik, 1976).

It has been demonstrated that for storage less than 1 wk at temperatures below physiological zero, reported to be 20 to 21°C, the embryo may remain in a state of embryonic diapause without harming

viability (Proudfoot and Hamilton, 1990). Funk and Biellier (1944) reported one of the few studies that observed the effects of a range of temperatures on the diameter of blastoderms. However limited recent research concerning the influence of environmental temperatures, between the time immediately following oviposition and the time when the fertile egg is cooled to below physiological zero, has been reported.

The specific objectives of the present study were to determine if preincubation embryonic development is influenced by 1) collecting eggs within 1 h of oviposition versus collecting eggs after they remained in the nest for 6 to 7 h, and 2) the method of egg storage during cooling. The relative significance of these two management factors was compared. The hypothesis tested was that embryos from eggs collected within 1 h after oviposition and cooled immediately would be less developed than embryos from eggs remaining in the nest all day. The two variables involved would be a reduced rate of cooling of eggs to physiological zero as a result of nest effects such as insulation provided by the litter and incubation by the hens, and egg storage effects due to the high insulation value of the egg packaging material.

MATERIALS AND METHODS

Stocks and Housing

Fertile eggs weighing between 55 and 65 g were collected from naturally inseminated Single Comb White Leghorn hens (Shaver Star Cross 288). The birds were housed in 3.8 x 4.8 m floor pens with a barn temperature of approximately 13°C, and a ratio of 7 roosters to 70 hens per

pen. Hens were 38 wk of age at the start of the experiment and 42 wk of age on the last day of egg collection. Access to 2 straw-bedded colony nests measuring 1.2 x .8 x .8 m was provided per pen. A typical layer diet containing 15% CP and 2800 kcal ME/kg was fed for *ad libitum* intake.

Experimental Design

The experiment consisted of two nest holding times and two cold storage methods in a 2 x 2 factorial arrangement of treatments. Eggs laid before 0800 h were removed from the nests and were not used in this experiment. Eggs laid between 0800 and 0900 h were collected over a period of 5 d, 1 d/wk for each of the 5 wk the experiment was ongoing. For the first 4 d of egg collection four pens were used. In addition to the four pens a further eight pens were used on the last day of collection to obtain a larger sample size. Eggs were randomly assigned to one of four treatment groups 1) Eggs, 0 to 1 h, unpacked. Freshly laid eggs were collected at 0900 h within 1 h of laying, placed on a 30-egg cardboard flat, and stored unstacked and unpacked. 2) Eggs, 0 to 1 h, packed. Freshly laid eggs were collected at 0900 h within 1 h of laying, and placed on a 30-egg cardboard flat. The flat holding the experimental eggs was stacked between four additional flats of freshly laid eggs, and stored inside a 30-dozen cardboard egg case with the lid closed. 3) Eggs, 6 to 7 h, unpacked. Freshly laid eggs were marked at 0900 h but remained in the nest until 1500 h when they were collected and handled as per Treatment 1. 4) Eggs, 6 to 7 h, packed. Freshly laid eggs were marked at 0900 h but remained in the nest until 1500 h when they were collected and handled as per Treatment 2. The eggs of all four treatment groups were stored at 13.8°C. The additional eggs used during the packing

procedure were not included in the data set for this experiment.

Embryo Staging

After 4 d of storage, eggs were broken open, and the albumen separated from the yolk. A filter paper ring was made by using a standard hole punch (6 mm in diameter) and cutting approximately 1 to 2 mm around the hole. The filter paper ring was placed on top of the vitelline membrane around the blastoderm to provide support while removing it from the surface of the yolk. The vitelline membrane was cut around the outside of the filter paper ring and the blastoderm lifted off the yolk. The blastoderm was placed ventral side up in a Petri dish containing physiological saline, and viewed under 160 magnification using a Wild M3 stereomicroscope[1] fitted with a Jena dark field illuminator[2]. Any adherent yolk on the blastoderm was removed using a hair loop or by gentle washing with a pipette.

Stage of embryonic development was assessed according to the method described by Eyal-Giladi and Kochav (1976). Any development past Stage 13, the final stage before primitive streak formation begins, was assigned a stage of 16 to facilitate statistical analysis.

Statistical Analyses

The data were analyzed for significance at the 5% level using analysis of variance. Sources of variation were replication (days of collection, d = 5), nest holding time (t = 2), method of storage (m = 2),

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[1]Leica Canada Inc., Willowdale, ON, Canada M2H 2C9.

[2]C.Z. Scientific Instruments (Canada) Inc., Markham, ON, Canada L3R 2L6.

and the interaction between nest holding time and method of storage. The interactions with replication was pooled to form the error term. The following model was used in the statistical analysis.

$$Y_{ijk} = \mu + D_i + H_i + M_j + HM_{ij} + e_{ijk}$$

where μ is the overall mean, H is the holding time, M is the method of storage, and e is the random error.

Contrasts within the HM interaction were computed to compare the methods of storage within each nest holding time, and to compare the nest holding time within each method of storage. Least square means (LSM) and standard error of means (SEM) were calculated. Computations were completed using the SAS® General Linear Model procedure (SAS Institute, 1985).

RESULTS

Two hundred and fifty embryos were assessed and grouped into Stages 10 through 13 and Stage 16 of embryonic development. Table II-1 presents the number of embryos per stage of development for each of the four treatment groups and the percentage of embryos in each stage per treatment group. A comparison of LSM of 0 to 1 h unpacked versus 0 to 1 h packed ($P = .0214$), 0 to 1 h unpacked versus 6 to 7 h unpacked ($P = .0001$), and 0 to 1 h packed versus 6 to 7 h packed ($P = .0152$) were significantly different, but 6 to 7 h unpacked versus 6 to 7 h packed ($P = .8595$) was not.

Also shown in Table II-1 are the numbers of embryos per stage of development, and the LSM for the two main treatment effects, collection

time and storage method, respectively. Embryos from eggs collected between 0 to 1 h were significantly less developed than embryos from eggs collected between 6 to 7 h after oviposition (P = .0001). However, condition of storage (P = .1140) and time by storage interaction (P = .0730) did not significantly affect stage of embryonic development.

DISCUSSION

In mammals that exhibit embryonic diapause or delayed implantation, cessation and reactivation of embryonic growth depends upon secretions from the endometrium into the uterine lumen (Aitken, 1977). Unlike mammals, development of the avian embryo ceases due to low environmental temperature exposure. Because the majority of embryonic development in the chicken occurs after oviposition, the environmental temperature to which the fertile egg is exposed plays a key role in embryonic growth rate. To prevent prolonged exposure to conditions in the poultry house, it has been recommended that for optimum embryo viability and thus hatchability, eggs should be collected at least four times daily (North, 1984).

In the present study, length of nest holding time significantly affected embryonic growth, possibly due to maternal warming. As the body temperature of the domestic hen (*Gallus domesticus*), reported to be 41.5°C (Whittow, 1986), is well above physiological zero, brooding by the hen will influence embryonic development. Eggs collected after 6 to 7 h had more of an opportunity to be incubated by hens than eggs collected within 0 to 1. This may be due to the different hens coming in and out of the

nest throughout the day to lay their eggs, and in the process, warming eggs which had been laid earlier. Thus embryos of eggs remaining for a longer period in the nest were significantly more developed due to the increased likelihood of inadvertent warming, or in some cases actual brooding by the hen. An increase in embryonic growth rate was not influenced by the environmental temperature, which was well below physiological zero, or the nest temperature, which was presumed to be below physiological zero. However, in warmer climates, environmental temperatures may be high enough for embryonic growth to continue.

Method of storage may also influence embryonic development by affecting the rate of egg cooling. North (1984) stated that it takes approximately 1 to 2 d to cool eggs from 32.2 to 18.3°C when eggs are stored in cases with holes in the sides, and .75 d when eggs are on incubator trays. These results indicate that exposure of the egg surface area to the environment affects rate of egg cooling.

In the present study, significance of method of storage varied with nest holding time. A comparison of the LSM of unpacked and packed eggs held in the nest for 0 to 1 h was significant, but unpacked and packed eggs held in the nest for 6 to 7 h was not. This may be due to two possible reasons. The first hypothesis is that at the time of storage, eggs collected after 0 to 1 h may have been warmer than eggs collected after 6 to 7 h because of a more recent oviposition time, and due to the inconsistent warming of the 6 to 7 h eggs by the hens as they entered the nest to lay eggs. Because of this, the 0 to 1 h packed eggs could have taken longer than the 6 to 7 h packed eggs to cool to below physiological zero, and thus the embryos of these eggs were exposed for a longer period

of time to the temperatures which promoted development. Another possible reason may be that at the time of storage, the embryos of 6 to 7 h packed eggs had developed to a point where only continuous incubation temperatures would promote further growth. The embryos of 0 to 1 h packed eggs may not have developed as far, and thus were still able to further develop under the temperatures provided by the possible reduced rate of cooling during packing.

Factors which affect the stage of embryonic development prior to storage may be important for a number of reasons. Investigators working with embryos prior to incubation, such as studies involved with the production of chimeric chicks, may require a specific stage of embryonic development. Through identification and regulation of the influencing factors, investigators may successfully regulate embryonic growth and thus obtain a higher ratio of embryos at the specific stage of embryonic development required.

Factors influencing prestorage embryonic development also warrant consideration as there may be an optimum stage at which the chicken embryo should be stored to maintain viability. Stockard (1921), observing embryonic diapause in the minnow *Fundulus heteroclitus*, determined that abnormalities are more likely to occur when embryo development is arrested prior to or during early gastrulation, or 1 day or more after gastrulation was complete. Stockard (1921) stated that if embryonic development was arrested during a period when no "rapid changes" were occurring, development may resume normally after proper conditions are provided for the embryo to grow. These periods that were not critical to embryo development he described as "moments of indifference". However, Stockard

(1921) also referred to "critical moments" in which rapid growth of the embryo is occurring and any interruption of growth would cause injury to the embryo. It has been postulated by Arora and Kosin (1966) that the extent of gastrulation at oviposition may influence the capability of the chick blastoderm to survive storage.

The results of the present study indicate that embryonic developmental rate is influenced by the amount of time the egg is held in the nest. The exposure of eggs in the nest boxes to frequent periods of maternal warming by the hens allowed incubation to take place. (In addition to the experimental eggs, approximately 5-10 eggs per nest box were also in the nest at 1500 h.) This was a key factor in this study as the environmental temperature was well below physiological zero and did not support embryonic growth. In geographical areas that have high environmental temperatures and lack temperature controlled housing, embryonic development may continue after oviposition irrespective of maternal incubation. Further identification of the factors that influence prestorage embryonic development is necessary, as there may be an optimum stage under which embryos should be stored to obtain optimum viability and hatchability.

TABLE II-1. Comparison for differences in embryonic development due to nest holding time and storage method.

Variable	Total number	Stages of embryo development					LSM[1]	SEM
		10	11	12	13	16		
Treatment								
1. 0 to 1 h Unpacked	65	39 (60.0%)[2]	8 (12.3%)	13 (20.0%)	5 (7.7%)	0 (0%)	10.76	.20
2. 0 to 1 h Packed	65	16 (24.6%)	13 (20.0%)	25 (38.5%)	11 (16.9%)	0 (0%)	11.52	.20
3. 6 to 7 h Unpacked	62	3 (4.8%)	9 (14.5%)	25 (39.7%)	22 (34.9%)	3 (4.8%)	12.41	.21
4. 6 to 7 h Packed	58	1 (1.7%)	7 (12.1%)	26 (44.8%)	22 (37.9%)	2 (3.4%)	12.36	.21
Main effects								
Nest holding time:								
0 to 1 h	130	55 (42.3%)	21 (16.2%)	38 (29.2%)	16 (12.3%)	0 (0%)	11.11[a]	.14
6 to 7 h	120	4 (3.3%)	16 (13.3%)	51 (42.5%)	44 (36.7%)	5 (4.2%)	12.33[b]	.15
Storage method:								
Unpacked	127	42 (33.1%)	17 (13.4%)	38 (29.9%)	27 (21.3%)	3 (2.4%)	11.51[a]	.15
Packed	123	17 (13.8%)	20 (16.3%)	51 (41.5%)	33 (26.8%)	2 (1.6%)	11.89[a]	.15

[a-b]Least square means with no common superscripts are significantly different for either of the main effects.

[1]Contrasts: 1) Treatment 1 versus Treatment 2 (P = .0214)
2) Treatment 1 versus Treatment 3 (P = .0001)
3) Treatment 2 versus Treatment 4 (P = .0152)
4) Treatment 3 versus Treatment 4 (P = .8595)

[2]Numbers of embryos in each stage per total number of embryos in each treatment x 100.

LITERATURE CITED

Aitken, R.J., 1977. Embryonic diapause. Pages 307-359 *in:* Development in Mammals 1. M.H. Johnson, ed. North Holland Publishing Company, Amsterdam, The Netherlands.

Arora, K.L., and I.L. Kosin., 1966. Developmental responses of early turkey and chicken embryos to pre-incubation holding of eggs: Inter- and Intra-species differences. Poultry Sci. 45:958-970.

Byng, A.J., and D. Nash, 1962. The effects of egg storage on hatchability. Br. Poult. Sci. 3-4:81-86.

Eyal-Giladi, H., and S. Kochav, 1976. From cleavage to primitive streak formation: A complementary normal table and a new look at the first stages of the development of the chick. I. General Morphology. Dev. Biol. 49:321-337.

Funk, E.M. and H.V. Biellier, 1944. The minimum temperature for embryonic development in the domestic fowl (*Gallus domesticus*). Poultry Sci. 23:538-540.

Kaplan, S., G.L. Kolesari, and J.P. Bahr, 1978. Temperature dynamics of the fertile chicken egg. Am. J. Physiol. 234:R183-R187.

North, M.O., 1984. Pages 72 and 77 *in:* Commercial Chicken Production Manual, 3rd ed. AVI Publishing Company, Westport, CT.

Olsen, M.W., and S.K. Haynes, 1948. The effect of different holding temperatures on the hatchability of hen's eggs. Poultry Sci. 27:420-425.

Proudfoot, F.G., 1966. Hatchability of stored chicken eggs as affected by daily turning during storage and prewarming and vacuuming eggs enclosed in plastic with nitrogen. Can. J. Anim. Sci. 46:47-50.

Proudfoot, F.G., and R.M.G. Hamilton, 1990. Care of Hatching Eggs Before Incubation. Publication 1573/E, Communications Branch, Agriculture Canada, Ottawa, ON, Canada.

Reinhart, B.S., and J.F. Hurnik, 1976. The effect of temperature and storage time during the pre-incubation period. 1. The influence of storage temperature changes on hatchability and first ten days chick performance. Poultry Sci. 55:1632-1640.

Romanoff, A.L., 1939. Effect of temperature shock on development of chick embryo. Pages 184-186 *in:* Seventh World's Poultry Congress and Exposition Proceedings. World's Poultry Science Association, ed. Waverly Press, Inc. Baltimore, MD.

Romijn, C., and W. Lokhorst. 1955. Chemical heat regulation in the chick embryo. Poultry Sci. 34:649-654.

SAS Institute, 1985. SAS® User's Guide. SAS Institute, Inc., Cary, NC.

Stockard, C.R., 1921. Developmental rate and structural expression: An experimental study of twins, "double monsters" and single deformities, and their interaction among embryonic organs during their origin and development. Am. J. Anat. 28:115-277.

Tretyakov, N.P., I.N. Zusman, L.J. Kiselev, 1973. Morphological variability in embryogenesis of the domestic fowl and factors affecting it. Poultry Sci. 52:93-101.

Whittow, G.C., 1986. Regulation of Body Temperature. Pages 221-268 *in:* Avian Physiology, 4th ed. P. D. Sturkie, ed. Springer-Verlag, New York, NY.

III. EFFECTS OF NEST HOLDING TIME DURING PERIODS OF HIGH ENVIRONMENTAL TEMPERATURE ON PREINCUBATION EMBRYO DEVELOPMENT, HATCHABILITY, AND EMBRYO VIABILITY IN BROILER BREEDERS

INTRODUCTION

The main factor which influences embryonic development in birds is the environmental temperature to which the egg is exposed (Edwards, 1902; Kaplan *et al.*, 1978). The chicken embryo functions as a poikilotherm up until the time of hatching (Romijn and Lokhorst, 1955). In temperatures above physiological zero (20 to 21°C), development of the embryo may continue (Proudfoot and Hamilton, 1990).

Previous work (Chapter II) demonstrated that embryos of eggs which remain in the nest for longer periods of time are significantly more developed than eggs which are collected within 1 h of oviposition. In the study described in Chapter II, the factor which influenced embryo development prior to storage was maternal incubation by the hen, and not environmental temperature, as the barn temperature was below physiological zero. However, in certain geographical areas, high environmental temperatures are common and are likely to influence embryo development if the temperature is above physiological zero.

Several studies have been conducted to investigate the effects of exposure of the hen to high environmental temperatures on fertility and hatchability (Heywang, 1944; El Jack and Kaltofen, 1969; Van Krey *et al.*, 1987). However, few studies regarding prolonged exposure of the egg to high environmental temperatures have been completed. El Jack and Kaltofen

(1969), determined that hatchability was significantly lower in eggs which were stored for 5 d at 38.3°C versus storage for 5 d at 15 or 29.5°C. A more detailed study by Heywang (1945) involved various frequencies of collection and methods of storage. Heywang (1945) concluded that eggs do not have to be gathered more than once a day, but should be stored in a cool place to maintain hatchability.

Conflicting results have been reported regarding the relationship between stage of embryonic development at oviposition and embryo viability. Prior to storage, it has been observed in chickens that embryos from strains with high hatchability had developed to further stages of gastrulation than embryos of birds with low hatchability (Nicolaides, 1933; Hays and Nicolaides, 1934). Inspection of chicken (Coleman and Siegel, 1966) and turkey embryos (Arora and Kosin, 1966) following storage has indicated that embryos in early stages of gastrulation may not be able to withstand storage as well as embryos which are more developed. However, Taylor and Gunns (1935), concluded that there is no correlation between size of the unincubated blastoderm and hatchability.

The specific objectives of the present study were to determine if there is an optimum stage of embryonic development at which to store eggs to obtain maximum embryo viability. The effects of collecting eggs during periods of high environmental temperatures within 1.5 h of oviposition, versus collecting eggs after they remained in the nest for 6 to 7 h, on preincubation embryo development and embryo viability were examined. The hypothesis was that embryos from eggs collected within 1 h after oviposition and cooled immediately would be less developed, which may

consequently influence viability.

MATERIALS AND METHODS

Stocks and Housing

During late June and early July of 1991, eggs were collected from two houses on two different commercial farms in Georgia. Broiler breeder hens (Hubbard) were naturally inseminated and housed in 12.2 x 114.3 m houses on farm 1, and 12.2 x 91.4 m houses on farm 2. At 20 wk, when the birds were placed, there were 7900 hens and 950 roosters per house on farm 1, and 6300 hens and 760 roosters per house on farm 2. The barns were curtain sided, and were equipped with evaporative cooling misters which were the only mechanisms providing temperature control. Two nest temperatures and one barn temperature were taken at the time of egg collection and egg handling in the barn. The age of the hens were 42 (farm 1) and 43 wk of age (farm 2). Taking mortality into consideration, during the experimental period the ratio of roosters to hens was approximately 9.5:1 on farm 1 and 11:1 on farm 2. Hens were provided access to two-tiered galvanized conventional nest boxes lined with shavings. A typical breeder diet containing 16% CP and 2865 kcal ME/kg was fed to the hens to maintain feed restriction at the level recommended by the breeder.

Experimental Design

A total of 16608 eggs were collected over a period of four days for each farm. On each day, 1038 eggs were collected from each barn for each of the two treatments, and a subsample of 30 eggs was set aside for embryo

development staging. The experiment consisted of two nest holding times. The nests were cleared of eggs by 0800 h. Eggs for Treatment 1 were collected between 0900 and 0930 h, within 1 to 1.5 h of oviposition, and placed in the coolers by 1000 h. Eggs for treatment 2 were gathered between 1100 and 1130 h, within 2.5 h of ovipostion, marked, and placed back in the nest by 1200 h. These eggs remained in the nests for a total of 6 to 7 h until collection at 1530 to 1600 h, and placement in the cooler at 1630. After collection, the eggs were placed into plastic incubator flats and stored on buggies for transport to the hatchery.

Embryo Staging

Immediately after collection, a subsample of eggs was used to assess stage of embryo development. Embryos were prepared for developmental staging as described previously (Chapter II). Stage of embryo development was assessed according to the method described by Eyal-Giladi and Kochav (1976). Any development past Stage 13, the last stage before primitive streak formation begins, was assigned a stage of 16 to facilitate statistical analysis.

Fertility, Hatchability, Viability, and Early Embryonic Deaths

Eggs to be sent to the commercial hatchery[1], were stored at the breeder farms at 18.9 to 20.7°C for 2 to 5 d. The eggs were incubated for 19 d in a Jamesway Super J incubator set at 37.2°C and 59% relative humidity. At 20 d the eggs were transfered to a Jamesway Super J hatcher set at 37.1°C and 60% relative humidity. After 21 d of incubation,

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[1]Gold Kist, Inc., Northwest Georgia Division, P.O. Box 747, Calhoun, GA 30703

hatchability (#eggs hatch/all eggs) was determined for each barn and treatment on each farm (1008 eggs). An estimate of fertility (#fertile eggs/all eggs), viability (#eggs hatch/#fertile eggs), and early embryonic deaths (died between 1 to 7 d of incubation) was also obtained for each house and treatment on each farm by completing a residual breakout after 21 d of incubation (672 eggs).

Statistical Analyses

The data were analyzed using analysis of variance (SAS Institute, 1990). Sources of variation were farms (f = 2), houses within farm (h = 2), days of collection within farm (d = 4), and treatments (t = 2). The interaction of treatment with the other identified sources of variation were pooled into the error term. Least square means and standard error of means were calculated. Significance was measured at $P < .05$.

RESULTS

Table III-1 presents the least square means, SEM, and number of observations for fertility, hatchability, viability, early dead embryos, stage of embryo development, nest temperature and barn temperature for each of the two nest holding times. Fertility (P = .8931), hatchability (P = .2795), viability (P = .4728), and early dead embryos (P = .4795) were not significantly affected by the two nest holding times. However, stage of embryo development (P = .0001), nest temperature (P = .0017), and barn temperature (P = .0001) were significantly affected by the nest holding time. Embryonic development was more advanced in eggs which remained in the nest for 6 to 7 h, versus eggs which were in the nests for

1 to 1.5 h. The nest and barn temperatures were higher during the period when the eggs were in the nest for 6 to 7 h.

DISCUSSION

In the present study fertility was not affected by the duration of the nest holding time. The observation that hatchability was not affected by the two nest holding times is supported by the work of Kirk *et al.* (1980) who determined that the hatchability of eggs collected within 1 h of oviposition, was not significantly different ($P < .10$) than eggs which remained in the nests for 5 h.

The increase in preincubation embryo development with increasing amount of time spent in the nest in the present study agrees with previous work (Chapter II) on Single Comb White Leghorns. A developmental stage of 10.76 reported for eggs in the nest for 0 to 1 h (Chapter II) is slightly higher than the value of 10.38 reported for eggs collected from 1 to 1.5 h in the present study. This may be indicative of a breed effect. The stage of development for eggs remaining in the nest for 6 to 7 h was 11.67 in the present study, and 11.52 in the previous study (Chapter II). The advanced stage of development may be a reflection of the high environmental temperature during the present study.

There are three factors which may have contributed to the increase in embryonic development viewed in the eggs that remained in the barn for 6 to 7 h. First, these eggs spent a greater amount of time in the barn exposed to temperatures which promoted embryonic development. Second, the average nest and barn temperatures were higher during the time which the

6 to 7 h eggs were in the barn, versus the period when the 1 to 1.5 h eggs were in the barn. This was due to the late afternoon gathering of eggs remaining in the nest for 6 to 7 h, versus the early morning collection of eggs in the nest for 1 to 1.5 h. As a result the rate of embryonic development in embryos of 6 to 7 h eggs collected during the afternoon may have been higher than the 1 to 1.5 h eggs collected in the morning. Third, the increasing time spent by the 6 to 7 h eggs in the nest provided a greater opportunity for maternal incubation to occur.

The results of the present study determined that nest holding time did not affect embryonic viability. Given the extensive literature relating the stage of embryonic development to embryo viability, it was somewhat unexpected that viability did not differ between the two nest holding treatments; especially since the stages of embryo development were significantly different. An analysis of early dead embryos was also non-significant between the two treatments. The results of the present study are supported by the early work of Taylor and Gunns (1935). It should be noted that the incidence of early dead embryos may have been underestimated, as early dead embryos could have been categorized as infertile eggs during the egg breakout after 21 d.

The results of this study indicate that although nest holding times during periods of high environmental temperatures affect preincubation embryo development, viability is not influenced by the stage of preincubation embryo development at storage. Factors such as egg sequence position, age and condition of the hen, and nutrition may be more crucial with respect to embryo viability.

TABLE III-1. Comparison for differences in fertility (# fertile eggs/# eggs set), hatchability (# eggs hatch/# eggs set), viability (# eggs hatch/# fertile eggs), early dead embryos (# dead between 1 to 7 d of incubation), and preincubation embryo development due to different nest holding times.

Treatment	Fertility (%)	Hatchability (%)	Viability (%)	Early dead embryos (%)	Stage of embryo development	Nest temp.[1] (°C)	Barn temp.[1] (°C)
1. Collected within 1 to 1.5 h of laying	95.88[a] ± .13[2] (10752)[3]	86.95[a] ± .07 (16128)	90.68[a] ± .16 (10752)	2.63[a] ± .12 (10752)	10.38[a] ± .04 (457)	28.06[a] ± .20 (16)	26.57[a] ± .07 (8)
2. Collected within 6 to 7 h of laying	95.85[a] ± .13 (10752)	86.73[a] ± .07 (16128)	90.49[a] ± .16 (10752)	2.79[a] ± .12 (10752)	11.67[b] ± .04 (453)	30.43[b] ± .20 (32)	29.87[b] ± .07 (16)

[a,b]Least square means within columns with no common superscripts are significantly different ($P < .05$).
[1]Nest and barn temperatures taken during time of collection for treatment 1 at 0900 h, and for treatment 2 at time of marking eggs and placing back in the nest at 1100 h, and at time of collection at 1530 h.
[2]SEM.
[3]Number of observations used in calculations.

LITERATURE CITED

Arora, K.L., and I.L. Kosin., 1966. Developmental responses of early turkey and chicken embryos to pre-incubation holding of eggs: Inter- and Intra-species differences. Poultry Sci.45:958-970.

Coleman, J.W., and P.B. Siegel, 1966. Selection for body weight at eight weeks of age. 5. Embryonic stage at oviposition and its relationship to hatchability. Poultry Sci. 45:1008-1011.

Edwards, C.L., 1902. The physiological zero and the index of development for the egg of the domestic fowl. Am. J. Physiol. 6:351-397.

El Jack, M.H., and R.S. Kaltofen, 1969. The effect of high holding and housing temperatures on hatchability of chicken eggs. Poultry Sci. 48:1013-1018.

Eyal-Giladi, H., and S. Kochav, 1976. From cleavage to primitive streak formation: A complementary normal table and a new look at the first stages of the development of the chick. I. General Morphology. Dev. Biol. 49:321-337.

Hays, F.A., and C. Nicolaides, 1934. Variability in development of fresh laid hen eggs. Poultry Sci. 13:74-89.

Heywang, B.W., 1944. Fertility and hatchability when the environmental temperature of chickens is high. Poultry Sci. 23:334-339.

Heywang, B.W., 1945. Gathering and storing hatching eggs during hot weather. Poultry Sci. 24:434-437.

Kaplan, S., G.L. Kolesari, and J. P. Bahr, 1978. Temperature dynamics of the fertile chicken egg. Am. J. Physiol. 234:R183-R187.

Kirk, S., G.C. Emmans, R. McDonald, and D. Arnot, 1980. Factors affecting the hatchability of eggs from broiler breeders. Br. Poult. Sci. 21:37-53.

Nicolaides, C.N., 1933. Relation of time of laying and embryonic mortality. Poultry Sci. 12:274-276.

Proudfoot, F.G., and R.M.G. Hamilton, 1990. Care of Hatching Eggs Before Incubation. Publication 1573/E, Communications Branch, Agriculture Canada, Ottawa, ON, Canada.

Romijn, C., and W. Lokhorst. 1955. Chemical heat regulation in the chick embryo. Poultry Sci. 34:649-654.

SAS Institute, 1990. SAS® User's Guide. SAS Institute, Inc., Cary, NC.

Taylor, L.W., and C.A. Gunns, 1935. Size of unincubated embryo in relation to hatchability. Poultry Sci. 14:294.

Van Krey, H.P., D.M. Denbow, and R. M. Hulet, 1987. The effect of ambient temperature on turkey embryonic development at oviposition. Theriogenology. 28:489-494.

IV. VARIABILITY IN PREINCUBATION EMBRYO DEVELOPMENT IN EGG-TYPE DOMESTIC FOWL. 2. EFFECTS OF DURATION OF EGG STORAGE PERIOD

INTRODUCTION

It is well documented that storing chicken eggs reduces embryo viability (hatch of fertile eggs) (Byng and Nash, 1962; Merritt, 1964; Mather and Laughlin, 1976, 1977; Whitehead *et al.*, 1985). Egg weight loss also increases as the number of days of storage is extended (Mather and Laughlin, 1976, 1977). Previous research indicates that during storage, embryonic development ceases when eggs are held at temperatures below physiological zero (20 to 21°C) (Edwards, 1902; Proudfoot and Hamilton, 1990). Further support for the cessation of embryonic development during storage is provided by Funk and Biellier (1944). Their study determined that there was "no discernible development of the blastoderm" below 27°C. However, Arora (1965), as cited by Mather and Laughlin (1977), demonstrated that during storage for 6 d at 12.8°C, blastoderm cells remain physiologically active. A hypothesis formulated by Kauffman (1948) states that different cells of the blastoderm may have distinct physiological zeros or optimum storage conditions.

Previous investigations have relied on blastoderm size as an indicator of embryonic development. During the present study the staging method described by Eyal-Giladi and Kochav (1976) was used to measure early embryo development. This method measures development microscopically on the basis of morphogenetic movements on the surface of the embryo, thus enabling more precise staging at earlier stages of embryo

development. The objective of this research was to confirm if embryo development ceases when fertile eggs are held below physiological zero. The hypothesis tested was that embryo development, although at a reduced rate during storage, may still occur, and therefore contribute to the decline in viability observed as days of storage increases. In addition, initial egg weight, and loss of egg weight during storage were measured to determine their relationship to embryo development and viability.

MATERIALS AND METHODS

Stocks and Housing

Eggs weighing between 45 to 65 g were collected from naturally inseminated Single Comb White Leghorn (Shaver) hens. The birds were housed in 30 m^2 floor pens with a room temperature of approximately 19°C, a nest temperature of 20°C, and a ratio of 12 roosters to 150 hens. Hens were 25 wk of age during the egg collection period. Each pen had four straw-bedded colony nests measuring 1.2 x .8 x .8 m. A typical layer diet containing 15% CP and 2800 kcal ME/kg was fed for *ad libitum* intake.

Experimental Design

The experiment involved collection of 700 eggs from six pens over a period of 5 d. The photoperiod was 14 h light:10 h dark with "lights on" at 0700 h. Each day 140 eggs laid between 0800 and 0900 h were gathered, placed on plastic egg flats small end down, and randomly divided into five groups of 28 eggs. The experimental groups were subjected to one of five lengths of storage; 0, 4, 7, 14, or 21 d. All eggs were stored at 14°C. This temperature was chosen as it has been recommended

that eggs be stored at 16 to 17°C for storage less than 7 d, and 11 to 12°C for storage greater than 7 d (Proudfoot and Hamilton, 1990). All eggs were weighed prior to, and immediately after storage.

Embryo Staging

After storage, the eggs were opened and fertility was determined. All infertile eggs and embryos which were damaged during the staging process were discarded. Blastoderms were removed from the yolk and viewed using the method described previously (Chapter II). Stage of embryo development was assessed according to the method characterized by Eyal-Giladi and Kochav (1976). Any development past stage 13, the last stage before primitive streak formation begins, was assigned a stage of 16 to facilitate statistical analysis.

Statistical Analysis

The data were analyzed using the General Linear Model Procedure (SAS Institute, 1990). Sources of variation were days of storage (d = 5). Least square means and SEM were calculated for loss of egg weight and embryonic development. The relationship between mortality (alive or dead) and length of storage (0, 4, 7, 14, 21) were analyzed using the chi square test of independence. Significance was measured at $P < .05$.

RESULTS

A total of 500 embryos, 100 per each storage period, were assessed and grouped into stages of embryonic development. Table IV-1 presents the least square means and SEM for loss of egg weight and stage of embryonic development, and the number of dead embryos for each storage period.

Analysis of covariance for stage of development indicated that date of collection (P = .5654), length of egg storage (P = .5815), and fresh egg weight (P = .3789) did not significantly affect stage of preincubation embryo development. A significant (P = .0001) linear relationship (b = .0742 ± .0010) between duration of egg storage and loss of egg weight was observed. Loss of egg weight was greater in eggs which were stored for longer periods.

DISCUSSION

The lack of a significant effect of egg storage on pre-incubation embryonic development corroborates the observations of Edwards (1902), Funk and Biellier (1944), and Proudfoot and Hamilton (1990), who determined that embryonic diapause occurs in fertile eggs which are stored below physiological zero. The work of Al-Muranni (1978) who demonstrated that, prior to 14 d of incubation, embryonic growth is independent of egg weight support the results of the present study.

Mather and Laughlin (1977) noted that the percent egg weight loss for storage after 7 and 14 d was 0.5 ± .34 (standard deviation) and 1.1 ± .43 respectively. The increase in percent egg weight loss with number of days of storage observed in the present study for 7 and 14 d at 1.2 and 2.1 respectively, was higher than the loss determined by Mather and Laughlin (1977). This may be attributed to differences in humidity, as Mather and Laughlin (1977) maintained humidity at 85%, while humidity was not controlled during storage in the present study. In addition, embryo mortality was significantly related to duration of storage (P = .0000).

Embryonic mortality was higher in eggs which were stored for longer periods, and had lost a greater amount of weight during storage. The observations of the present study are supported by previous research in turkeys (Arora and Kosin, 1966a) and in chickens (Mather and Laughlin, 1976; Reinhart and Hurnik, 1976; Mather and Laughlin, 1977) which demonstrated that the incidence of abnormal and dead embryos significantly increased as storage time increased. The incidence of vacuoles in the germinal area of degenerating blastoderms has been reported in detail by Arora and Kosin (1966b). In the present study it was observed that dead embryos had weak vitelline membranes which were partially disintegrated or tore easily, or had numerous vacuoles in the area pellucida and area opaca. In addition, it was observed that there were many mottled yolks in eggs stored for 14 and 21 d versus eggs which were stored at 0, 4, or 7 d.

Using the more precise method of embryo staging developed by Eyal-Giladi and Kochav (1976), it was confirmed that preincubation embryo development ceases when eggs are stored at temperatures below physiological zero. The increase in embryonic mortality viewed as duration of storage increases corroborates previous work mentioned in this study. Experiments regarding the degeneration of egg quality as storage time progresses need to be completed as there may be a relationship between egg quality and embryo viability.

TABLE IV-1. Fresh egg weight, loss of egg weight, embryonic mortality, and embryonic development in eggs varying in duration of storage.

Duration of egg storage (d)	Fresh egg weight (g)[1]	Loss of egg weight (g)[2]	Number of dead embryos (/100)[3]	Mean stage of embryonic development[4]
0	55.48 ± .28	0 ± .01	0	9.90 ± .04
4	55.31 ± .29	.47 ± .02	3	9.97 ± .04
7	54.56 ± .28	.66 ± .02	2	9.93 ± .04
14	54.73 ± .30	1.17 ± .02	12	9.93 ± .05
21	54.94 ± .31	1.57 ± .02	18	9.89 ± .05

[1]Least square means and SEM. Embryonic development not affected by fresh egg weight (P = .3789).
[2]Least square means and SEM. Loss of egg weight linearly related (b = .0742 ± .0010) to length of egg storage (P = .0001).
[3]Embryonic mortality related to length of egg storage (P = .0000).
[4]Least square means and SEM. Embryonic development not affected by length of egg storage (P = .5815).

LITERATURE CITED

Al-Murrani, W.K., 1978. Maternal effects on embryonic and post-embryonic growth in poultry. Br. Poult. Sci. 19:277-281.

Arora, K.L., and I.L. Kosin., 1966a. Developmental responses of early turkey and chicken embryos to preincubation holding of eggs: Inter- and intra-species differences. Poultry Sci. 45:958-970.

Arora, K.L., and I.L. Kosin., 1966b. Changes in the gross morphological appearance of chicken and turkey blastoderms during preincubation storage. Poultry Sci. 66:819-825.

Byng, A.J., and D. Nash, 1962. The effects of egg storage on hatchability. Br. Poult. Sci. 3-4:81-86.

Edwards, C.L., 1902. The physiological zero and the index of development for the egg of the domestic fowl. Am. J. Physiol. 6:351-397.

Eyal-Giladi, H., and S. Kochav, 1976. From cleavage to primitive streak formation: A complementary normal table and a new look at the first stages of the development of the chick. I. General Morphology. Dev. Biol. 49:321-337.

Funk, E.M., and H.V. Biellier, 1944. The minimum temperature for embryonic development in the domestic fowl (*Gallus domesticus*). Poultry Sci. 23:538-540.

Kauffman, L., 1948. The effect of certain thermic factors on the morphogenesis of fowl embryos. Pages 351-356 *in*: Official Report of the Eighth World's Poultry Congress. World's Poultry Science Association, ed. J. Baelum and A. Nielsen, Nielsen and Lydiche, Copenhagen, Denmark.

Mather, C.M., and K.F. Laughlin, 1976. Storage of hatching eggs: The effect on total incubation period. Br. Poult. Sci. 17:471-479.

Mather, C.M., and K.F. Laughlin, 1977. Storage of hatching eggs: The effect on early embryonic development. Brit. Poult. Sci. 18:597-603.

Merritt, E.S. 1964. Pre-Incubation storage effects on subsequent performance of chickens. Br. Poult. Sci. 5:67-73.

Proudfoot, F.G., and R.M.G. Hamilton, 1990. Care of hatching eggs before incubation. Publication 1573/E, Communications Branch, Agriculture Canada, Ottawa, ON, Canada.

Reinhart, B.S., J.F. Hurnik, 1976. The effect of temperature and storage time during the pre-incubation period. 1. The influence of storage changes on hatchability and first ten days chick performance. Poultry Sci. 55:1632-1640.

SAS Institute, 1990. SAS® User's Guide. SAS Institute, Inc., Cary, NC.

Whitehead, C.C., M.H. Maxwell, R.A. Pearson, and K.M. Herron, 1985. Influence of egg storage on hatchability, embryonic development and vitamin status in hatching broiler chicks. Br. Poult. Sci. 26:221-228.

V. RELATIONSHIP OF HEN AGE AND EGG SEQUENCE POSITION WITH FERTILITY, HATCHABILITY, VIABILITY, AND PREINCUBATION EMBRYO DEVELOPMENT IN BROILER BREEDERS.

INTRODUCTION

It is well established that as hens age, egg production (Atwood, 1929; Bahr and Palmer, 1989; Etches, 1990; Robinson *et al.*, 1990), fertility, and hatchability (Kirk *et al.*, 1980) decline. In reviewing the literature, Bahr and Palmer (1989) reported that the age-related decline in egg production, viewed as the laying of fewer but larger eggs, may be due to an increased incidence of atresia and internal laying, a decrease in recruitment of follicles into the hierarchy, a reduction in shell quality, and/or a decrease in ovulation rate. One result of a decline in ovulation rate is a decrease in sequence length (Atwood, 1929; Robinson *et al.*, 1990) and hence an increase in the incidence of "first of sequence" eggs.

In the domestic hen egg laying occurs in sequences of one or more eggs, separated by non-laying days called "pause days" (Etches, 1990). "First of sequence" eggs are laid immediately after a pause day. Early investigators studying the ovulatory cycle initially hypothesized that first of sequence eggs remained in the oviduct, specifically the shell gland, during the pause day (Warren and Scott, 1935). However, after further study, Scott and Warren (1936) concluded that as the first egg of the clutch could not be detected in the oviduct until 20.4 h after the laying of the previous egg, the "day of non-laying" must be due to a delay

in ovulation. The most mature follicle (F1) which may become a first of sequence egg takes approximately 40 h to be laid from the time of oviposition of the previous egg, versus about 24 h for other eggs in a sequence (Etches, 1990). As discovered by Scott and Warren (1936), the mature F1 follicle from first of sequence eggs remains on the ovary for about 16 h longer than the mature F1 follicle of subsequent eggs. It has been hypothesized that the extended time that the first of sequence egg spends on the ovary may be detrimental to fertility and hatchability, and may influence embryonic development at oviposition.

A recent study which characterized sequence length in feed restricted broiler breeder hens determined that early in the production cycle, sequence length is short, reaches a prime mean sequence length at 32 wk of age, then quickly shortens with advancing hen age (Robinson *et al.*, 1990). This indicates there are more pause days and more first of sequence eggs early and late in the hens life, than during the mid portion of the laying cycle (Robinson *et al.*, 1990). The high incidence of first of sequence eggs during these times coincides with less than optimum values for fertility, hatchability, and viability.

Several studies have been completed on the effects of egg sequence position on embryo and chick production parameters. Kosin and Mun (1960) studying the effects of clutch size in turkeys found that eggs of multiple egg clutches had significantly higher fertility and hatchability than eggs of single egg clutches. Bacon and Nestor (1979) conducting research on medium and large weight turkey hens, determined that first of sequence eggs had significantly lower fertility, hatchability, and hatch of fertile eggs, and a higher incidence of embryonic mortality at 7 d of incubation,

than did subsequent eggs. In contrast, Robinson *et al.* (1991), investigating the effects in broiler breeders, observed no significant differences in fertility or viability at 7 d of incubation between first and subsequent eggs of a sequence. However they did determine that embryo production, or the number of viable embryos per 100 eggs set (fertility x viability), was significantly reduced in first of sequence eggs when compared to subsequent eggs. This research was completed over a period of 45 d beginning at 45 wk of age. During this time period, the hens were in the middle of their laying cycle and the incidence of first of sequence eggs was low.

The focus of the present study was to follow a group of hens through a longer period of the egg production cycle so that more first of sequence eggs could be analyzed. The objectives were to determine if hen aging and egg sequence position are significantly related to fertility, hatchability, viability and preincubation embryo development. In addition, hen weight, egg weight, shell weight, time of oviposition, and number of days since insemination were monitored to determine their relationship to the egg production parameters measured.

MATERIALS AND METHODS

Stocks and Housing

An experiment was conducted using 30 Indian River broiler breeder hens from 31 to 54 wk of age. One hen died at 44 wk, therefore data from this hen was deleted from the data set. The birds were housed in individual laying cages in a light tight facility, and placed on a

photoperiod regimen of 14 h light:10 h dark. Room temperature was maintained at 18 to 19°C. A paddle mounted on each of the egg collection trays of the cages was connected to a movement sensitive mercury switch, which was linked to a computer. This enabled data collection on the exact time of oviposition. Each hen was artificially inseminated once per week, with 50 μL of fresh pooled semen collected from 60 broiler breeder males of the same age as the females. A typical broiler breeder diet of 15.5% CP and 2,850 kcal ME/kg was fed to obtain modest body weight gains throughout the breeding period, as recommended by the breeder.

Experimental Design

Egg production was recorded daily over a 24 wk period. Eggs were collected hourly from 0800 h to 1700 h. At the time of collection, the eggs were weighed, placed on plastic egg flats, and stored at 16 to 17°C for .5 to 7 d. The eggs were separated into one of two experimental groups.

1) Fertility and Preincubation Embryo Development. During the 12 odd numbered weeks, eggs were evaluated for stage of embryo development. At the time of embryo development staging, egg weight was recorded, and fertility assessed after opening the egg. Shell weight was measured after 2 d of air drying at room temperature. The embryo was lifted off the yolk and viewed according to the method described previously (Chapter II). Stage of embryonic development was assessed using the method characterized by Eyal-Giladi and Kochav (1976). Any development past stage 13, the last stage before primitive streak formation begins, was assigned a stage of 16 to facilitate statistical analysis.

2) Fertility, Hatchability, and Embryo Viability. During the 12

even numbered weeks eggs were sent to a commercial hatchery[1] and incubated for 17 d in a Jamesway Big J incubator set at 37.2°C and 86% relative humidity. At 18 d the eggs were transfered to a Jamesway Big J hatcher set at 37.1°C and 86% relative humidity. After 21 d of incubation, any unhatched eggs were opened and fertility and stage of embryonic mortality assessed. From the data, hatchability (# eggs hatch/# eggs set x 100), and viability (# eggs hatch/# fertile eggs x 100) were determined.

The fertility data from both experimental groups were pooled to determine fertility (# fertile/all eggs x 100). For both experimental groups, soft shelled and double-yolked eggs were not included in the data set, but were recorded to facilitate assigning eggs to a sequence position. Using oviposition records, each egg was assigned to being a "first" or "subsequent" egg of a sequence. Eggs which were laid immediately after a pause day were classified as first, while all other eggs in a sequence were catagorized as subsequent. Hen age (wk), hen weight (kg), egg weight (g), number of days since insemination (2-8), and time of oviposition (h) were also recorded during both experiments.

Statistical Analyses

Stepwise logistic regression analysis (SAS Institute, 1990) was used in analyzing the data for the production parameters fertility, hatchability, and viability (recorded at two levels i.e. fertile or infertile) (Glantz and Slinker, 1990). Stepwise regression analysis (SAS Institute, 1990) was used for the quantitative parameter, embryo development. The variables included in the regression were egg sequence

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[1]Lilydale Co-operative Hatchery, Edmonton, Alberta, Canada, T5E 5T1.

position, hen age, hen weight, egg weight, number of days since insemination, and time of oviposition. A probability value of $P < .05$ was required for entry of a variable into the equations.

Overall mean values for the 29 hens were computed for each of the variables which entered into the regression equations for fertility, hatchability, viability, and preincubation embryo development. Standard error of proportions were calculated for the production parameters analyzed by logistic regression as outlined in Byrkit (1987). SEM were calculated for embryonic development.

RESULTS

The significant variables that entered into the regression equations are presented in Table V-1 according to order of entry. Hen weight, number of days since insemination, hen age, and egg weight were significantly related to fertility, while all of the above factors, excluding egg weight, were related to hatchability. In addition to number of days since insemination and hen weight, egg sequence position demonstrated a significant relationship with viability. The factors exhibiting a significant relationship with stage of embryonic development at oviposition were shell weight, egg sequence position, oviposition time, hen weight, and number of days since insemination.

The different levels of variable means for the production parameters and for embryo development are presented in Table V-2, so that the effect of the variables on the production parameters can be observed. A trend of decreasing fertility and hatchability was noted as hen age increased.

Viability was lower and embryonic development more advanced in first versus subsequent eggs of a sequence. As the number of days since insemination increased, fertility, hatchability, and viability all decreased. Although there was no consistent trend with each successive day past the insemination date, a general trend of decreasing embryonic development was observed. After excluding the lowest hen weight class, which had a small number of observations, a trend of decreasing fertility, hatchability, viability, and embryonic development was noted as hen weight increased. Excluding the lower and upper egg weight classes with small numbers of observations, fertility decreased as egg weight increased. Embryonic development was more advanced as shell weight increased and as oviposition time increased from 0800 to 1400 h.

DISCUSSION

A relationship between hatchability and sequence position approached significance (P = .0573), however, fertility and hatchability were not significantly related to sequence position. This agrees with previous work with broiler breeders (Robinson *et al.*, 1991), but contradicts the results obtained by Bacon and Nestor (1979) in turkeys. Species differences may play a role in this instance.

In the present study, hen age was significantly related to fertility and hatchability. Kirk *et al.* (1980) observed that fertility declined approximately 11% from 34 to 60 wk of hen age which agrees with the approximate 10% reduction in fertility from 31 to 54 wk noted in the present study. Kirk *et al.* (1980) also noted that hatchability declined

from a peak hatchability of 91% at 44 wk to 82% at 60 wk of age. Although the decline in hatchability started at an earlier age, the results of the present research project show a similar decrease from 92% at 31 to 34 wk to 80% at 51 to 54 wk. This reduction in fertility and hatchability observed with increasing hen age may be due to a decline in the ability of older hens to retain spermatozoa in the uterovaginal sperm host gland (UV-SHG) as observed in turkeys (Van Krey *et al.*, 1967; Christensen, 1981) and broiler breeders (Pierson *et al.*, 1988a), or a lower quality follicle which may not be fertilized, or may not survive to hatching. It is well documented in mammals (see Adams, 1975; Mikamo and Hamaguchi, 1975 for review) and amphibians (see Mikamo and Hamaguchi, 1975 for review) that with advancing maternal age, fertility declines and the incidence of chromosomal anomalies increases.

The relationship between hen weight and fertility and hatchability was significant. The two production parameters decreased as hen weight increased. During this investigation the birds were fed amounts of feed concomitent with a modest weight gain during the production period. Therefore a consistent increase in hen weight was a reflection of hen aging, which was previously described as significantly reducing fertility and hatchability.

Age of the spermatozoa, as reflected by an increase in number of days since insemination, was related to fertility and hatchability. A consistent downward trend in fertility was observed early after insemination, starting on the third day, which agrees with the work of Lodge *et al.* (1971). Their research determined that fertility consistently declined beginning 5 days after insemination. In addition,

Nalbandov and Card (1943) concluded that hatchability decreases with an increase in the "staleness" of sperm. The cause of the decline in fertility and hatchability may be due to a successive decrease in the number of sperm in the UV-SHG with increasing days past insemination (Pierson *et al.*, 1988b), or an inability of older sperm to fertilize the ovum as has been demonstrated in chickens (Nalbandov and Card, 1943) and in mammals (see Mann and Lutwak-Mann, 1975 for review).

Another variable which increases with hen age is egg weight, which is mainly due to an increase in yolk deposition (Bahr and Palmer, 1989). There was a significant relationship between egg weight and fertility. As egg weight increased from a range of 55-65 g to a range of 65-75 g fertility decreased. This may be explained by two hypotheses. First, as hens age they lay fewer but larger eggs (Bahr and Palmer, 1989) due to the reduction in follicular maturation. This suggests that the follicle could be subject to pre-ovulatory aging which may reduce oocyte quality and negatively influence the capability of the follicle to be fertilized. Second, a reduction in follicular maturation also reduces sequence length. Although there was no significant relationship between sequence position and fertility in the present study, the increased incidence of first of sequence eggs may still have contributed to the decline in fertility observed with increasing egg weight. First of sequence eggs are heavier than subsequent eggs (Atwood, 1929; Robinson *et al.*, 1990) and are more susceptible to preovulatory aging.

Although the variables presented in Table V-1 were significantly related to embryonic development, the R value obtained was .27 indicating much unexplained variation for this parameter. It is hypothesized that

the major factor affecting embryo development is the environmental temperature to which the egg is exposed (Chapter II and III).

A significant relationship with hen age was not demonstrated, however sequence position was related to viability and embryonic development. Embryos of first of sequence eggs were not as viable, and were more developed than embryos of subsequent eggs. Earlier research projects have observed a decrease in viability (Bacon and Nestor, 1979), and embryo production (Robinson *et al.*, 1991) in first versus subsequent eggs of a sequence.

The most common stage of embryonic development at oviposition is a stage of 10 (Eyal-Giladi and Kochav, 1976). In the present study a more advanced stage of embryonic development was observed in first of sequence eggs. Mather and Laughlin (1979) hypothesized that embryos develop further as the hen ages due to an increased incidence of first of sequence eggs laid by older hens. In addition, McNally and Byerly (1936) determined that embryos from eggs laid early in the morning had more somites after 48 h of incubation than eggs laid in the afternoon. Although they did not state this was due to egg sequence position, as first of sequence eggs are laid early in the morning it may be postulated that a large proportion of the eggs being analyzed were first of sequence.

Hen weight was significantly related to both viability and embryonic development. It has been previously mentioned that due to the feeding regimen in the present study, hen weight increased with hen age. It was observed that viability was high, and embryonic development was consistent with what is usually observed at oviposition (Eyal-Giladi and Kochav, 1976) when the hens were young, and body weight was low. As the hens aged

and body weight increased, viability and embryonic development increased, then both gradually declined. These results are consistant with the work of Tomhave (1956) who determined that hatch of fertile eggs decreases with increasing age of the hen.

Viability and embryonic development were related to the number of days since insemination. A downward trend in viability was observed as the number of days since insemination increased. A high incidence of chromosomal anomalies in embryos fertilized by aged sperm has been reported in mammals (see Shaver and Martin-DeLeon, 1975 for review); and in chickens, studies have shown an increase in embryonic death (Lodge *et al.*, 1971) and abnormal embryos (Dharmarajan, 1950) with sperm aging. Although a consistent downward trend in embryonic development was not observed, there was a general trend of decreasing development as days since insemination increased. This is in agreement with the results of Nalbandov and Card (1943) who determined that embryonic development decreased as "staleness of sperm" increased.

The observation that embryonic development was related to shell weight was somewhat unexpected. A trend of increasing embryonic development was noted as shell weight increased. The relationship between a heavy shell weight and a more advanced embryo may be due to the effect of first of sequence eggs. Choi *et al.* (1985) established that the high incidence of heavy eggs, with heavy shells, laid in the morning was due to a greater proportion of first of sequence eggs laid during this time of day.

A significant relationship between oviposition time and embryonic development was observed in the present study. Eggs laid early in the

morning were slightly more developed than that which is usually observed at oviposition. Hutt and Pilkey (1929) and McNally and Byerly (1936) reported that embryos from eggs laid early in the morning were more developed than embryos from eggs laid later in the day. This is likely due to the high incidence of first of sequence eggs which are laid in the morning. The decline in embryonic development observed as oviposition time increased from early to mid-morning, may be explained by the greater proportion of subsequent eggs which are laid during this time period. A increased incidence of last eggs of a sequence, which are oviposited late in the day, may explain the trend of increasing embryonic development with increasing oviposition time after mid-morning. Mather and Laughlin (1979) established that embryos of first and last eggs of a sequence are more developed than embryos of mid-sequence eggs.

Previous investigators have attempted to associate the stage of embryonic development at oviposition to viability. Hays and Nicolaides (1934) noted that embryos from hens with low hatchability were at pregastrula or early gastrula stages of development, while embryos from hens with high hatchability were at well advanced stages of gastrulation. An extensive study by Funk (1934) determined that peak hatchability occured in eggs which were laid between 1200 h and 1400 h, which is when many intermediate eggs of a sequence are laid. The data from the present research indicates that the developmentally advanced embryos of first of sequence eggs were less viable than embryos of subsequent eggs of a sequence. However, from these results it can not be conclusively stated that a more developed embryo, than that which is usually observed at oviposition, has a reduced chance of survival. Because of the extended

period of time that the follicle of a first of sequence egg spends on the ovary, there may be follicle aging effects or yolk compositional differences which contribute to the increase in embryonic mortality. Further studies on follicle aging, and yolk compositional differences in domestic fowl, need to be completed to better understand the factors influencing the reduction in viability observed in first of sequence eggs.

TABLE V-1. Variables included in stepwise regression of fertility, hatchability, embryo viability, and preincubation embryo development[1].

Parameters	n[2]	Variables[3]	Significance*
Fertility	3240	1. Hen weight	(.0001)
		2. Days since insemination	(.0001)
		3. Hen age	(.0001)
		4. Egg weight	(.0001)
Hatchability	1653	1. Days since insemination	(.0001)
		2. Hen weight	(.0001)
		3. Hen age	(.0002)
Viability	1487	1. Egg sequence position	(.0026)
		2. Days since insemination	(.0049)
		3. Hen weight	(.0240)
Embryo Development	1200	1. Shell weight	(.0001)
		2. Egg sequence position	(.0001)
		3. Oviposition time	(.0001)
		4. Hen weight	(.0022)
		5. Days since insemination	(.0430)

[1]Stepwise logistic regression for fertility, hatchability and viability, and stepwise linear regression for preincubation embryo development.
[2]Number of eggs assessed for each parameter.
[3]Listed according to order of entry into equation.
*Probability for inclusion into stepwise regression.

TABLE V-2. Values for production parameters for the levels of the variables.

Variable	Production Parameters			
	Fertility	Hatchability	Embryo Viability	Embryo Development
Overall:	90.68% ± .51[1] (3240)[2]	85.18% ± .87[1](1553)	94.82% ± .58[1](1467)	10.14 ± .03[3](1200)
Sequence Position:				
First	88.88% ± 1.00 (980)	81.39% ± 1.73 (505)	91.93% ± 1.29 (446)	10.36 ± .06 (339)
Subsequent	91.46% ± .59 (2260)	86.85% ± 1.00(1041)	95.77% ± .62(1041)	10.05 ± .03 (861)
Hen Age (wk):				
31-34	93.21% ± .99 (648)	92.45% ± 1.48 (318)	96.71% ± 1.02 (304)	10.18 ± .07 (261)
35-38	93.77% ± 1.00 (578)	88.62% ± 1.86 (290)	96.62% ± 1.10 (266)	9.95 ± .06 (226)
39-42	93.94% ± 1.01 (561)	85.81% ± 2.05 (289)	94.27% ± 1.44 (262)	10.13 ± .08 (229)
43-46	91.23% ± 1.22 (536)	82.37% ± 2.28 (278)	93.65% ± 1.54 (244)	10.09 ± .05 (190)
47-50	85.99% ± 1.91 (464)	79.17% ± 2.62 (240)	91.79% ± 1.91 (207)	10.12 ± .08 (154)
51-54	83.22% ± 1.76 (453)	79.83% ± 2.60 (238)	93.14% ± 1.77 (204)	10.39 ± .08 (140)
Days since insemination:				
2	94.67% ± 1.04 (469)	92.44% ± 1.71 (238)	97.35% ± 1.07 (226)	10.26 ± .07 (195)
3	94.88% ± 1.02 (469)	89.71% ± 1.95 (243)	95.18% ± 1.42 (228)	10.17 ± .07 (178)
4	92.95% ± 1.20 (454)	90.76% ± 1.86 (238)	95.58% ± 1.37 (228)	10.09 ± .08 (150)
5	90.93% ± 1.35 (452)	84.58% ± 2.40 (227)	93.66% ± 1.70 (205)	10.11 ± .08 (161)
6	90.58% ± 1.35 (467)	85.59% ± 2.29 (236)	95.28% ± 1.46 (212)	10.07 ± .07 (175)
7	87.04% ± 1.52 (486)	79.18% ± 2.59 (245)	93.72% ± 1.59 (207)	10.14 ± .08 (161)
8	83.52% ± 1.76 (443)	73.45% ± 2.94 (226)	90.71% ± 2.14 (183)	10.04 ± .08 (150)
Hen weight (kg):				
2.2 ≤ x ≤ 2.7	92.66% ± 2.50 (109)	86.67% ± 4.39 (60)	94.55% ± 3.06 (55)	10.07 ± .12 (42)
2.7 < x ≤ 3.2	93.74% ± .83 (847)	89.33% ± 1.54 (403)	95.74% ± 1.04 (376)	10.21 ± .06 (357)
3.2 < x ≤ 3.7	92.05% ± .70 (1472)	87.73% ± 1.18 (766)	95.58% ± .78 (702)	10.15 ± .05 (545)
3.7 < x ≤ 4.2	84.73% ± 1.25 (812)	76.42% ± 2.06 (424)	91.53% ± 1.48 (354)	10.00 ± .05 (256)
Egg weight (g):				
45 ≤ x ≤ 55	88.00% ± 4.50 (50)	87.50% ± 8.27 (15)	100.00% ± .00 (14)	10.14 ± .21 (22)
55 < x ≤ 65	91.56% ± .66 (1751)	86.84% ± 1.14 (874)	95.35% ± .75 (796)	10.05 ± .04 (675)
65 < x ≤ 75	89.47% ± .82 (1415)	83.67% ± 1.35 (753)	94.16% ± .91 (666)	10.25 ± .04 (490)
75 < x ≤ 86	95.83% ± 4.08 (24)	50.00% ± 15.91 (10)	55.56% ± 16.56 (9)	10.15 ± .22 (13)
Shell weight (g):				
2 ≤ x ≤ 3.5	100.00% ± .00 (7)	-	-	9.33 ± .33 (3)
3.5 < x ≤5	90.25% ± 1.93 (236)	-	-	9.91 ± .07 (184)
5 < x ≤ 6.5	91.25% ± .81 (1223)	-	-	10.17 ± .03 (920)
6.5 < x ≤ 8	94.78% ± 2.07 (115)	-	-	10.48 ± .13 (93)
Oviposition time (h):				
6 ≤ x ≤ 8	85.71% ± 2.82 (154)	83.99% ± 3.05 (149)	91.91% ± 2.34 (136)	10.21 ± .12 (99)
8 < x ≤ 10	90.91% ± .86 (1111)	85.51% ± 1.59 (490)	94.80% ± 1.06 (442)	9.99 ± .05 (362)
10 < x ≤ 12	91.95% ± .89 (932)	88.09% ± 1.49 (470)	96.50% ± .89 (428)	10.06 ± .04 (368)
12 < x ≤ 14	90.10% ± 1.30 (525)	82.64% ± 2.23 (288)	93.33% ± 1.56 (255)	10.22 ± .06 (171)
14 < x or 6 > x	89.96% ± 1.32 (519)	82.91% ± 2.36 (258)	93.91% ± 1.50 (226)	10.42 ± .09 (200)

[1] Standard error of proportions
[2] Number of eggs assessed
[3] SEM

LITERATURE CITED

Adams, C.E., 1975. Effects of maternal age on ovulation, fertilization and embryonic development. Pages 231-248 *in*: Aging Gametes, Their Biology and Pathology. R. J. Blandau, ed. S. Karger, Basel, Switzerland.

Atwood, H. 1929. Observations concerning the time factor in egg production. Poultry Sci. 8:137-140.

Bacon, W.L. and K.E. Nestor, 1979. Reproductive traits of first eggs of clutches vs. other clutch positions in turkeys. Poultry Sci. 58:257-258.

Bahr, J.M., and S.S. Palmer, 1989. The influence of aging on ovarian function. Crit. Rev. Poult. Biol. 2:103-110.

Byrkit, D.R., 1987. Pages 305-306 *in*: Statistics Today. A Comprehensive Introduction. The Benjamin/Cummings Publishing Company, Inc. Menlo Park, CA.

Christensen, V.L., 1981. Effect of insemination intervals on oviducal sperm storage in turkeys. Poultry Sci. 60:2150-2156.

Choi, J.H., R.D. Miles, A. S. Arafa, and R. H. Harms, 1981. The influence of oviposition time on egg weight, shell quality, and blood phosphorus. Poultry Sci. 60:824-828.

Dharmarajan, M. 1950. Effect on the embryo of staleness of the sperm at the time of fertilization in the domestic hen. Nature 165:398.

Etches, R.J., 1990. The ovulatory cycle of the hen. Critical Reviews in Poultry Biology. 2:293-318.

Eyal-Giladi, H., and S. Kochav, 1976. From cleavage to primitive streak formation: A complementary normal table and a new look at the first stages of the development of the chick. I. General Morphology. Dev. Biol. 49:321-337.

Funk, E.M., 1934. Relation of time of laying to hatchability. Poultry Sci. 34:184-187.

Glantz, S.A., and B.K. Slinker, 1990. Pages 546-566 *in*: Primer of Applied Regression and Analysis of Variance. McGraw-Hill, Inc. New York, NY.

Hays, F.A., and C. Nicolaides, 1934. Variability in development of fresh-laid hen eggs. Poultry Sci. 13:74-90.

Hutt, F.B., and A.M. Pilkey, 1929. Studies in embryonic mortality in the fowl. IV. Comparative mortality rates in eggs laid at different periods of the day and their bearing on theories of the origin of monsters. Poultry Sci. 9:194-203.

Kirk, S., G.C. Emmans, R. McDonald, and D. Arnot, 1980. Factors affecting the hatchability of eggs from broiler breeders. Br. Poult. Sci. 21:37-53.

Kosin, I.L., and A.M. Mun, 1960. Clutch size, oviposition time, and "floor" eggs as factors in turkey hatchability. Poultry Sci. 39:82-92.

Lodge, J.R., N.S. Fechheimer, and R.G. Jaap, 1971. The relationship of *in vivo* sperm storage interval to fertility and embryonic survival in the chicken. Biol. Reprod. 5:252-257.

Mann, T., C. Lutwak-Mann, 1975. Biochemical aspects of aging in spermatozoa in relation to motility and fertilizing ability. Pages 122-150 *in*: Aging Gametes, Their Biology and Pathology. R. J. Blandau, ed. S. Karger, Basel, Switzerland.

Mather, C.M., and K.F. Laughlin, 1979. Storage of hatching eggs: The interaction between parental age and early embryonic development. Brit. Poult. Sci. 20:595-604.

McNally, E.H., and T.C. Byerly, 1936. Variation in the development of embryos of hens' eggs. Poultry Sci. 15:280-283.

Mikamo, K., and H. Hamaguchi, 1975. Chromosomal disorder caused by preovulatory overripeness of oocytes. Pages 72-97 *in*: Aging Gametes, Their Biology and Pathology. R. J. Blandau, ed. S. Karger, Basel, Switzerland.

Nalbandov, A., and L.E. Card, 1943. Effect of stale sperm on fertility and hatchability of chicken eggs. Poultry Sci. 22:218-226.

Pierson, E.E., L.M. Krista, and G. R. McDaniel, 1988a. Effect of age and physiological status on sperm storage 24 hours after artificial insemination in broiler breeder hens. Brit. Poult. Sci. 29:193-197.

Pierson, E.E., G.R. McDaniel, and L.M. Krista, 1988b. Relationship between fertility duration and *in vivo* sperm storage in broiler breeder hens. Brit. Poult. Sci. 29:199-203.

Robinson, F.E., R.T. Hardin, and A.R. Robblee, 1990. Reproductive senescence in domestic fowl: egg production, sequence length and inter-sequence pause length. Brit. Poult. Sci. 31:871-879.

Robinson, F.E., R.T. Hardin, and B.J. Williams, 1991. The influence of egg sequence position on fertility, embryo viability, and embryo weight in broiler breeders. Poultry Sci. 70:760-765.

SAS Institute, 1990. SAS® User's Guide. SAS Institute, Inc., Cary, NC.

Scott, H.M., and D.C. Warren, 1936. Influence of ovulation rate on the tendency of the fowl to produce eggs in clutches. Poultry Sci. 15:381-389.

Shaver, E.L., and P.A. Martin-DeLeon, 1975. Effects of aging of sperm in the female and male reproductive tracts before fertilization on the chromosome complement of the blastocysts. Pages 151-165 *in*: Aging Gametes, Their Biology and Pathology. R. J. Blandau, ed. S. Karger, Basel, Switzerland.

Tomhave, A.E., 1956. Influence of age of New Hampshire female breeders upon hatchability of eggs. Poultry Sci. 35:236-237.

Van Krey, H.P., A.T. Leighton, Jr., and L.M. Potter, 1967. Sperm gland populations and late seasonal declines in fertility. Poultry Sci.46:1332.

Warren, D.C., and H.M. Scott, 1935. The time factor in egg formation. Poultry Sci. 14:195-207.

VI. GENERAL DISCUSSION

The experiments conducted in this thesis were completed because of the limited amount of knowledge available regarding factors which affect preincubation, early embryonic growth in domestic fowl, and the relationship between stage of embryo development and embryo viability. Although it is recognized that nutritional and genetic factors may also influence embryo development, the focus in this thesis was on environmental and physiological factors. The specific factors tested to determine the effects on embryonic development, were nest holding time during cool and hot environmental temperatures, method of egg storage, length of egg storage, egg sequence position, and hen age. In addition, concurrent fertility, hatchability and viability studies were conducted in experiments in chapters III and V.

Many initial investigations of early embryonic growth and development used the size of the blastoderm (Taylor and Gunns, 1935; Coleman and Siegel, 1966; Tretyakov *et al.*, 1973) or the number of somites (McNally and Byerly, 1936) as an indication of embryo growth. In addition, it is presumed that most of the early investigators believed that the onset of hypoblast formation signaled the beginning of gastulation, as it was thought that hypoblast cells formed the endoderm layer in the embryo (see Rol'nik, 1970; Romanoff, 1960 for review). Therefore it is difficult to determine what stage of embryonic development these investigators were observing when they referred to early, mid, and late gastrulation, as no definitions of gastrulation were given in the manuscripts describing the experiments. Recent investigations of

embryonic growth in domestic fowl have relied on weighing the embryo after various days of incubation as an indication of growth (Al-Murrani, 1978; Bacon and Nestor, 1979; Robinson, *et al.*, 1991). Using this method, it is difficult to relate embryonic growth to before incubation, as it is likely there are factors during incubation which misconstrue the actual stage of development prior to incubation.

The experiments conducted in this thesis used the early embryonic staging technique developed by Eyal-Giladi and Kochav (1976). This method categorizes embryonic growth into distinct stages through direct microscopic observation of cells of the early embryo. This provides the investigator with a repeatable, precise method for developmental staging of the preincubation embryo.

Through the use of the Eyal-Giladi and Kochav (1976) staging method it was determined that preincubation embryonic growth was not influenced by method of storage, but was affected by the length of time an egg is held in the nest.

Due to the differences in embryonic development observed with nest holding time in chapter II, it was postulated that the reduction in hatchability observed in geographical areas where the environmental temperature is high, may be due to the embryos being stored at advanced stages of development. Since there have been many studies completed associating the stage of embryonic development at oviposition (Nicolaides, 1933; Hays and Nicolaides, 1934) it was hypothesized that there may be an optimum stage of embryonic development at which to store eggs to maintain hatchability and embryo viability. The evidence obtained in the study completed in chapter III indicates that the hatchability potential of an

egg is not influenced by the stage of embryonic development at the time of storage. However, it can not be conclusively stated that the stage of embryonic development does not affect embryonic viability because of the limitations of the experimental method; the eggs which were staged were not the same eggs on which hatchability was determined. As the technique has not yet been developed for which large quantities of eggs can be staged for embryonic development and then hatched, "sister" eggs were used to test the hypothesis. The limitations of this experimental method are recognized.

Previous investigators have stated that all embryonic development ceases during storage at temperatures below physiological zero (Edwards, 1902; Funk and Biellier, 1944; Proudfoot and Hamilton, 1990). However, these investigators have relied on methods of measuring embryonic growth which were less precise than the Eyal-Giladi and Kochav (1976) method of staging. In chapter IV, the hypothesis tested was that embryos of eggs stored for long periods of time may continue to develop, although at a reduced rate. It was postulated that reduced rates of embryonic development could be viewed fairly easily, as the Eyal-Giladi and Kochav method (1976) stages embryonic growth in terms of hours rather than days. The results from this study concur with the observations of previous investigators that embryonic development does indeed cease at temperatures below physiological zero. The increase in embryonic mortality observed as storage time increased was linearly related to the loss of the egg weight. This leads to the hypothesis that embryonic mortality may increase due to a reduction in egg quality as the storage time increases.

The final experiment completed in this thesis (chapter V) tested the

hypothesis that the decline in fertility, and hatchability observed as hens age, may be due to the increased incidence of first of sequence eggs which are seen as hens age. However, the results demonstrated that although fertility and hatchability declined with age, sequence position was not related to this decline. This indicates that there may be a more general decline in reproductive function and quality of all follicles as the hen ages, rather than just with first of sequence eggs. From this experiment it was also observed that first of sequence eggs had higher stages of embryonic development, but lower viability than subsequent eggs. In this case it appears as though embryonic development is related to viability. However, because first of sequence eggs are more likely to be affected by follicle aging, the effects of follicle aging on egg quality must be tested, before it can be conclusively stated that the reduced embryo viability observed is due to a more developed embryo at storage.

The results of this thesis have identified problems and new questions which need to be addressed. One of the technique problems which needs to be solved is the ability to stage the early embryonic development of an egg, and then incubate it until hatching. Once this problem is solved the relationship between stage of embryonic development at the time of oviposition and storage, and embryo viablity may be conclusively answered. In addition, it is evident that egg quality as it relates to embryonic development and viability needs to be addressed. Determining the effects of egg storage and egg sequence position on egg quality, and subsequent embryonic development and viability, should be objectives for future studies.

LITERATURE CITED

Al-Murrani, W.K., 1978. Maternal effects on embryonic growth and post-embryonic growth in poultry. Br. Poult. Sci. 19:277-281.

Bacon, W.L., and K.E. Nestor, 1979. Reproductive traits of first eggs of clutches vs. other clutch positions in turkeys. Poultry Sci. 58:257-258.

Coleman, J.W., and P.B. Siegel, 1966. Selection for body weight at eight weeks of age. 5. Embryonic stage at oviposition and its relationship to hatchability. Poultry Sci. 45:1008-1011.

Edwards, C.L., 1902. The physiological zero and the index of development for the egg of the domestic fowl. Am. J. Physiol. 6:351-397.

Eyal-Giladi, H., and S. Kochav, 1976. From cleavage to primitive streak formation: A complementary normal table and a new look at the first stages of the development of the chick. I. General Morphology. Dev. Biol. 49:321-337.

Funk, E.M., and H.V. Biellier, 1944. The minimum temperature for embryonic development in the domestic fowl (*Gallus domesticus*). Poultry Sci. 23:538-540.

Hays, F.A., and C. Nicolaides, 1934. Variability in development of fresh-laid hen eggs. Poultry Sci. 13:74-90.

McNally, E.H., and T.C. Byerly, 1936. Variation in the development of embryos of hens' eggs. Poultry Sci. 15:280-283.

Nicolaides, C.N., 1933. Relation of time of laying and embryonic mortality. Poultry Sci. 12:274-276.

Proudfoot, F.G., and R.M.G. Hamilton, 1990. Care of Hatching Eggs Before Incubation. Publication 1573/E, Communications Branch, Agriculture Canada, Ottawa, ON, Canada.

Robinson, F.E., R.T. Hardin, N.A. Robinson, B.J. Williams, 1991. The influence of egg sequence position on fertility, embryo viability, and embryo weight in broiler breeders. Poultry Sci. 70:760-765.

Rol'nik, V.V., 1970. Pages 85-87 *in*: Bird Embryology. Israel Program for Scientific Translations, Ltd. Jerusalem, Israel.

Romanoff, A.L., 1960. Pages 160-165 *in*: The Avian Embryo. Structural and Functional Development. The Macmillan Company, New York, NY.

Taylor, L.W., and C.A. Gunns, 1935. Size of unincubated embryo in relation to hatchability. Poultry Sci. 14:294.

Tretyakov, N.P., I.N. Zusman, L.J. Kiselev, 1973. Morphological variability in embryogenesis of the domestic fowl and factors affecting it. Poultry Sci. 52:93-101.

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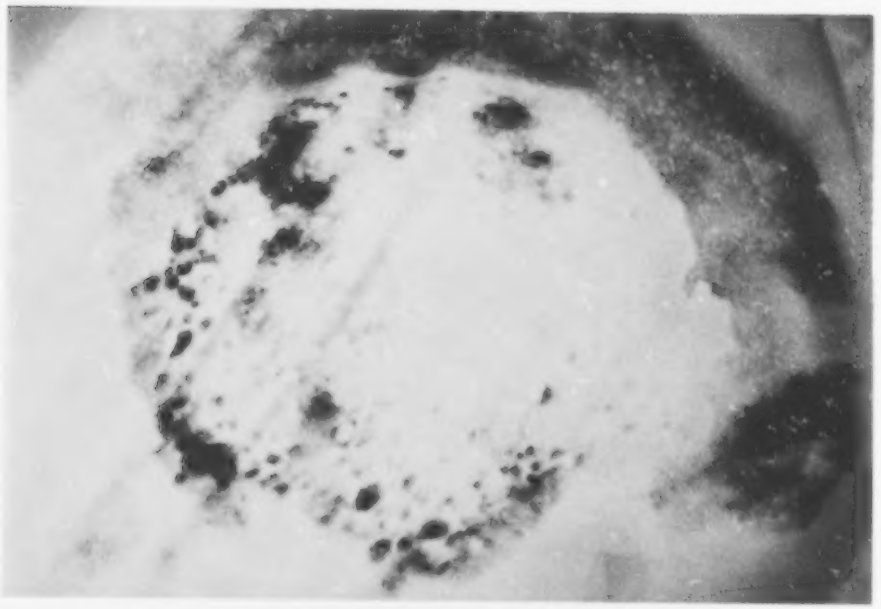


Plate II-1. Infertile germ - 160 magnification.

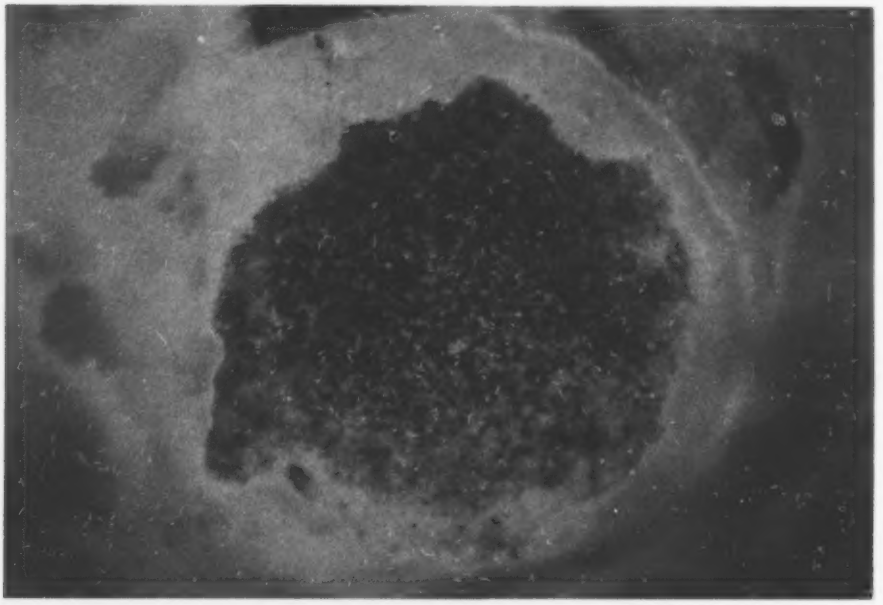


Plate II-2. Stage 10 Eyal-Giladi and Kochav (1976) - 160 magnification.

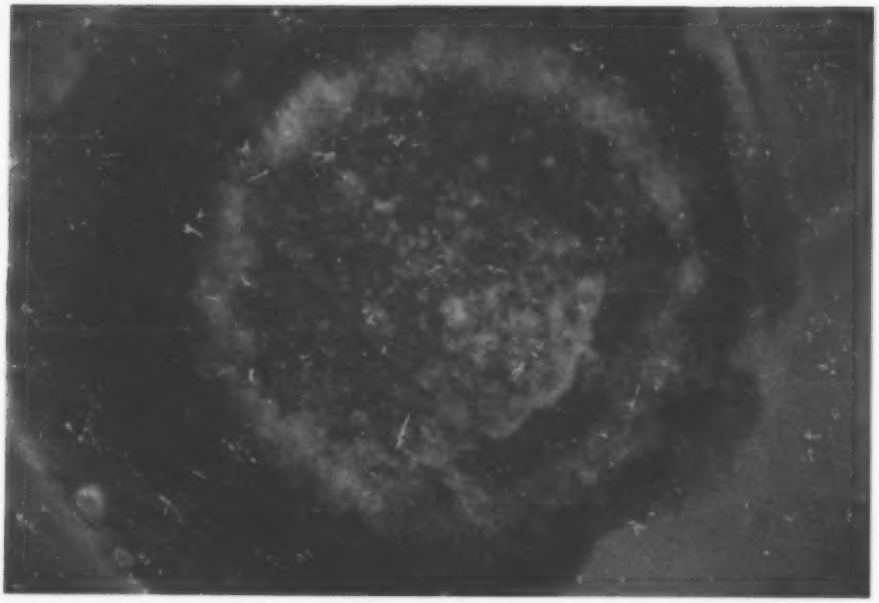


Plate II-3. Stage 11 Eyal-Giladi and Kochav (1976) - 160 magnification.

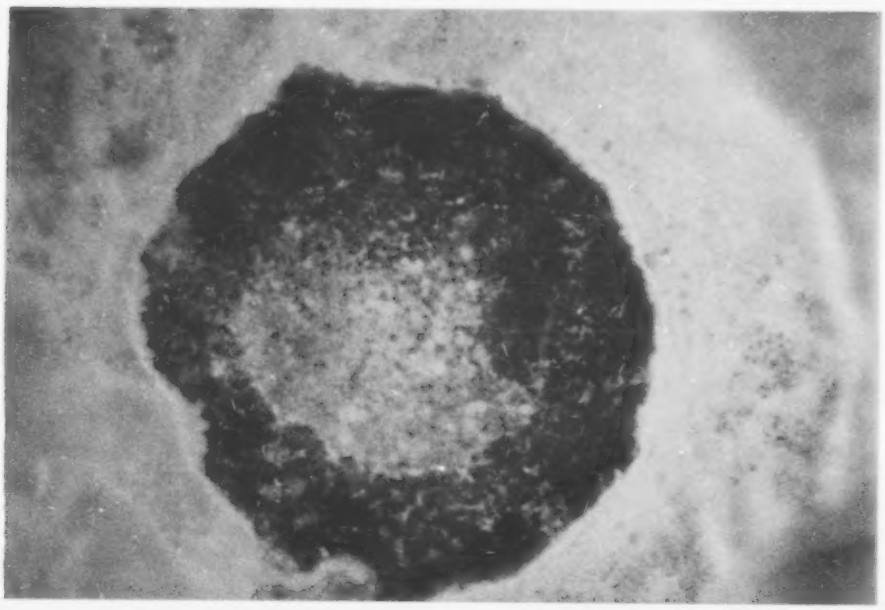


Plate II-4. Stage 12 Eyal-Giladi and Kochav (1976) - 160 magnification.

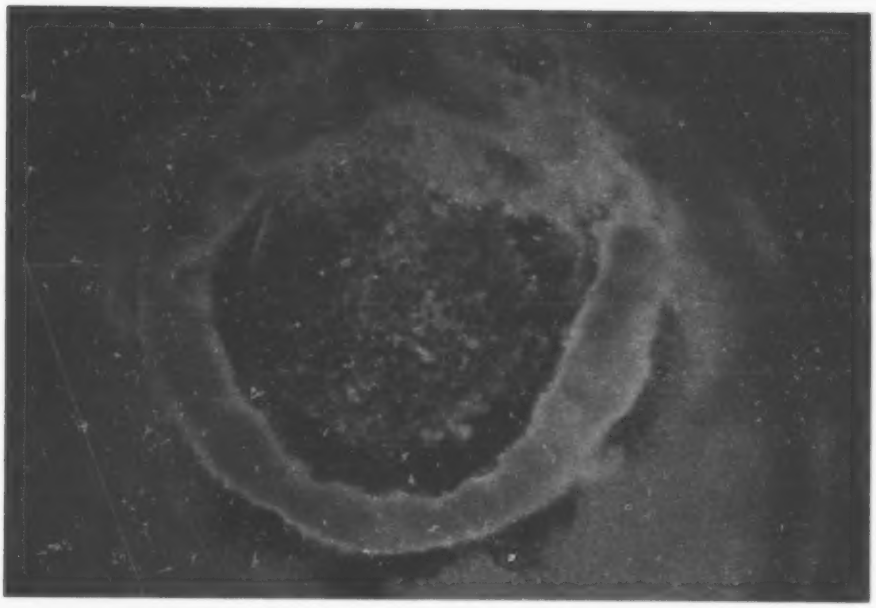


Plate II-5. Stage 13 Eyal-Giladi and Kochav (1976) - 160 magnification.

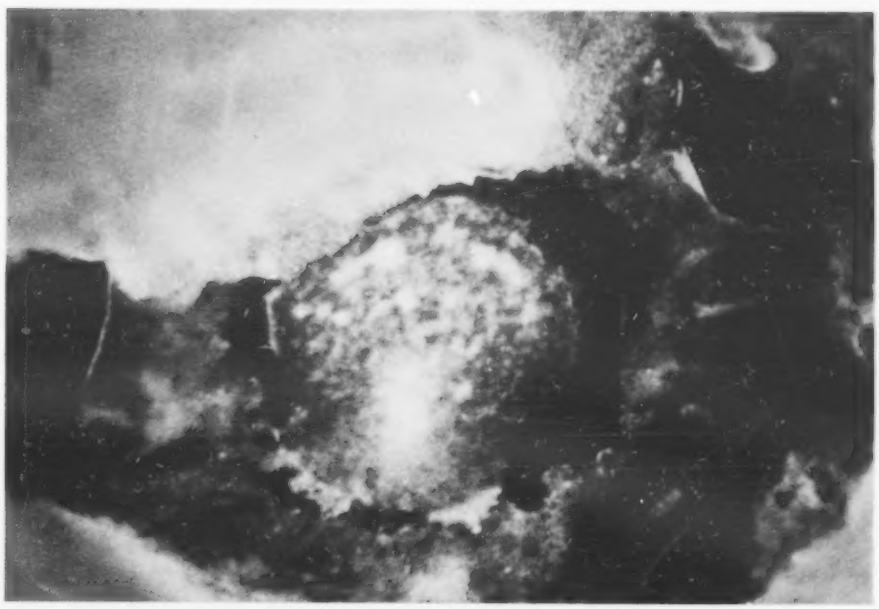
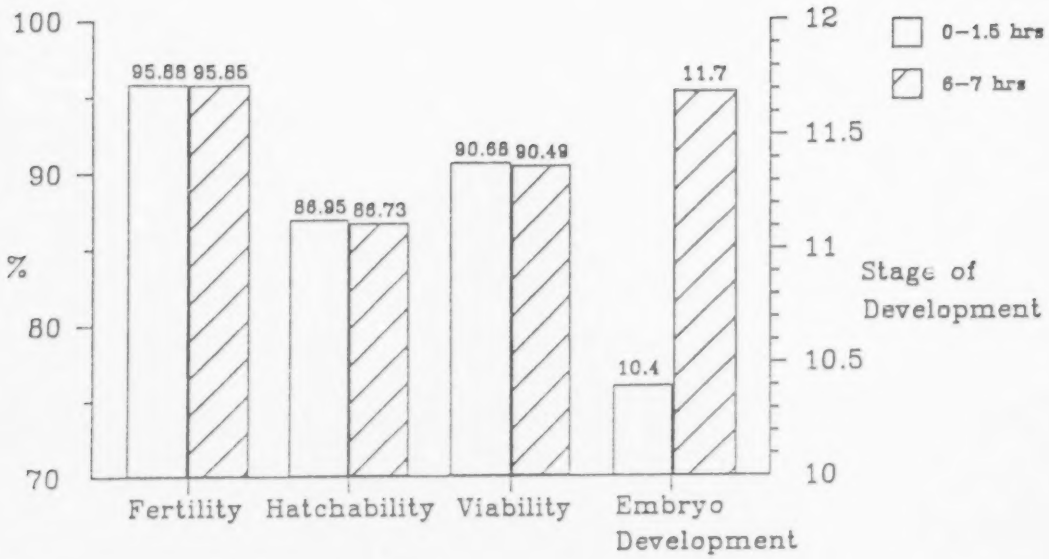


Plate II-6. Stage 3 Hamburger and Hamilton (1951) - 160 magnification.



	nest temp.(°C)	barn temp.(°C)
0-1.5 hrs	28.1	26.6
6-7 hrs	30.4	29.9

Figure III-1. Fertility, hatchability, viability (% - left y axis) and preincubation embryo development (Eyal-Giladi and Kochav (1976) - right y axis) of eggs remaining in nests for 0-1.5 hours or 6-7 hours during high environmental temperatures.

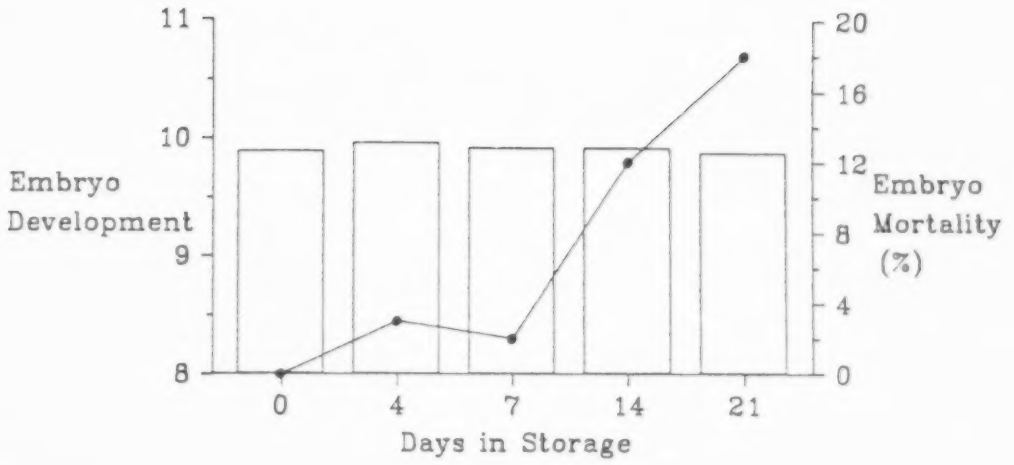


Figure IV-1. Comparison of preincubation embryo development (Eyal-Giladi and Kochav (1976) - left y axis) with embryo mortality (% - right y axis) for embryos of eggs stored for 0, 4, 7, 14, or 21 days (x axis).

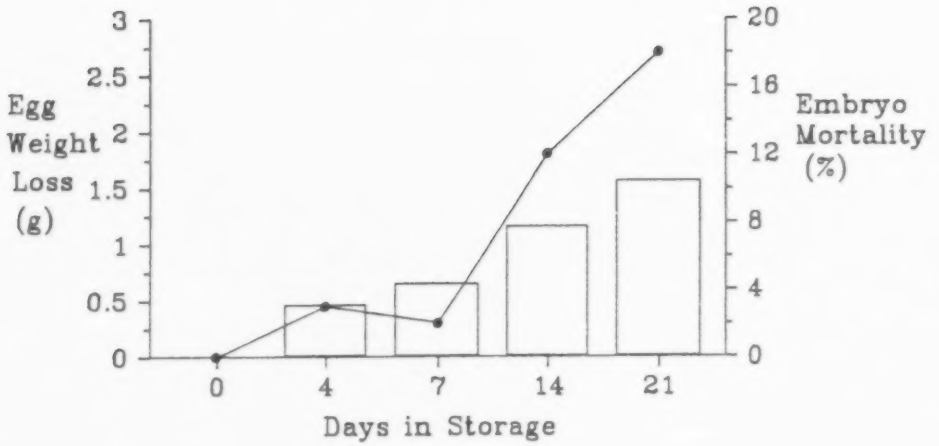


Figure IV-2. Comparison of egg weight loss (grams - left y axis) with embryo mortality (% - right y axis) for eggs stored for 0, 4, 7, 14, or 21 days (x axis).

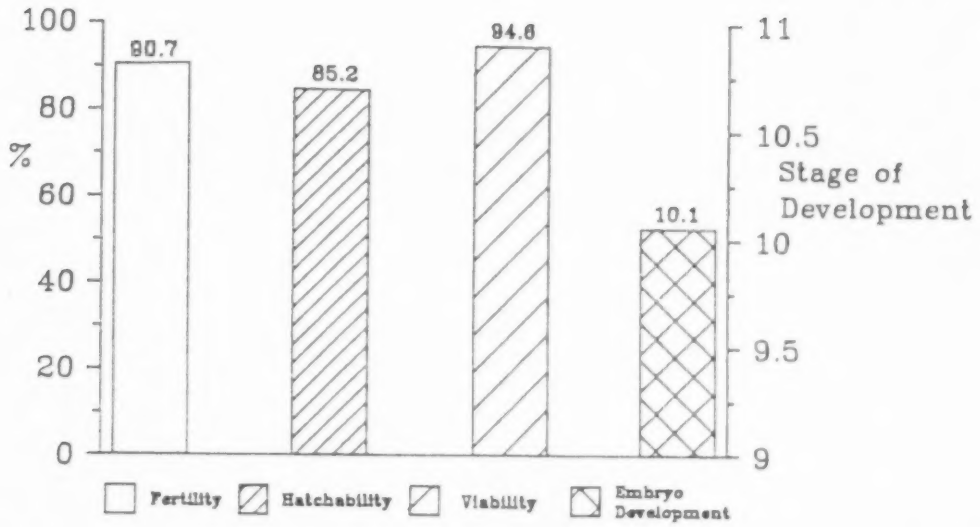


Figure V-1. Overall values for fertility, hatchability, viability, (% - left y axis) and preincubation embryo development (Eyal-Giladi and Kochav (1976) - right y axis) for 29 hens during the period of 31-54 wk of hen age.

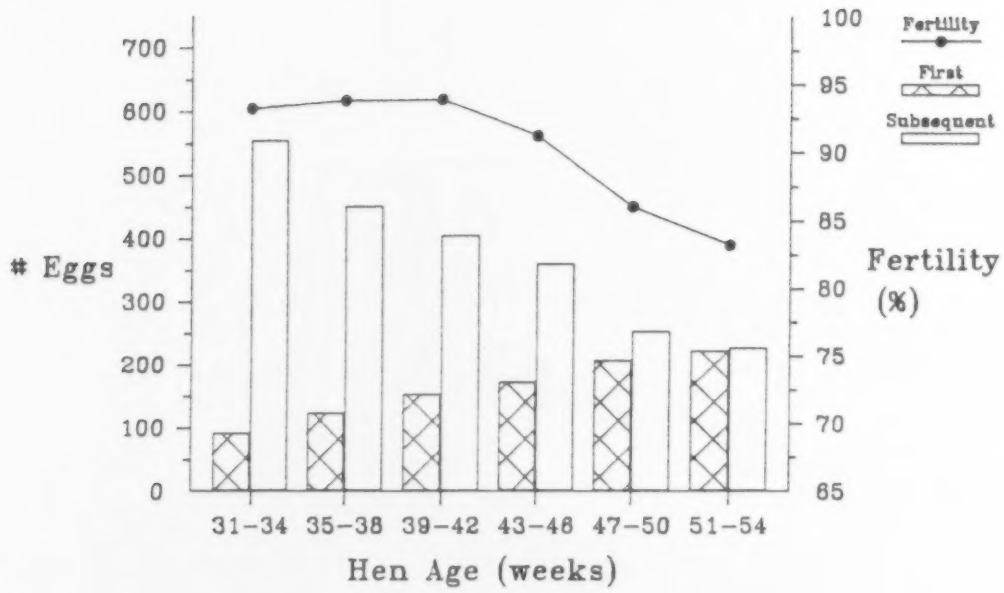


Figure V-2. Comparison of fertility (% - right y axis) with number of first and subsequent egg of a sequence (left y axis) and hen age (wk - x axis).

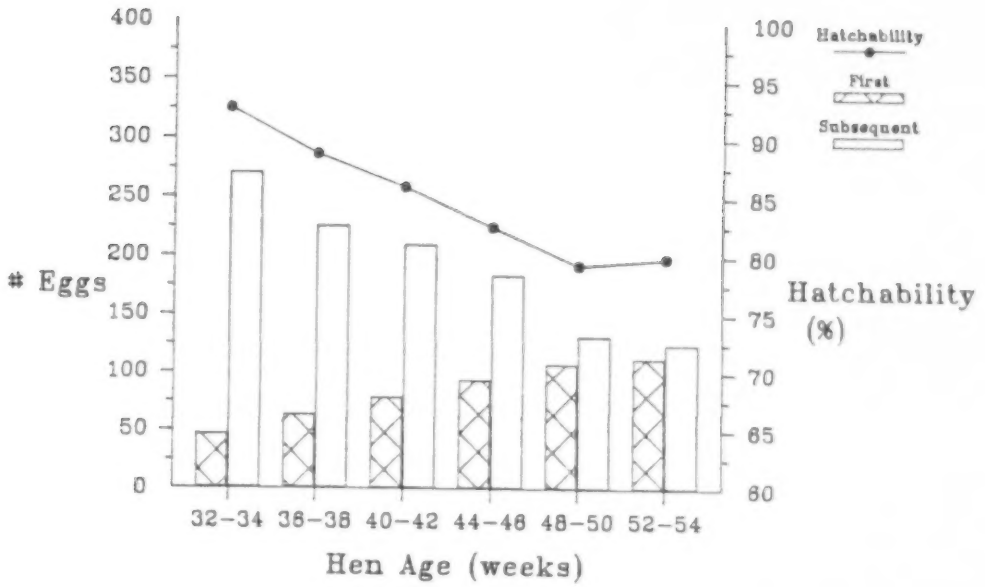


Figure V-3. Comparison of hatchability (% - right y axis) with number of first and subsequent egg of a sequence (left y axis) and hen age (wk - x axis).

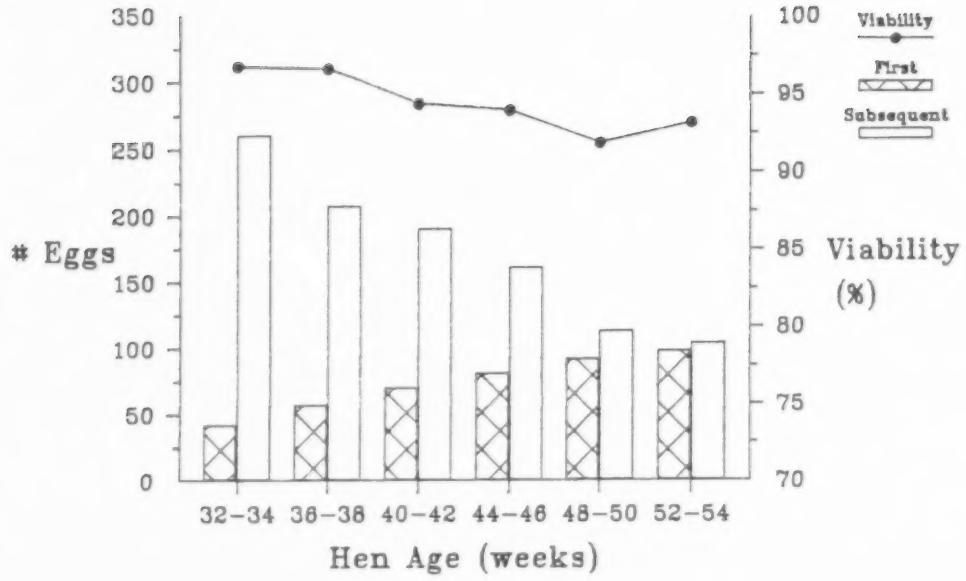


Figure V-4. Comparison of viability (% - right y axis) with number of first and subsequent egg of a sequence (left y axis) and hen age (wk - x axis).

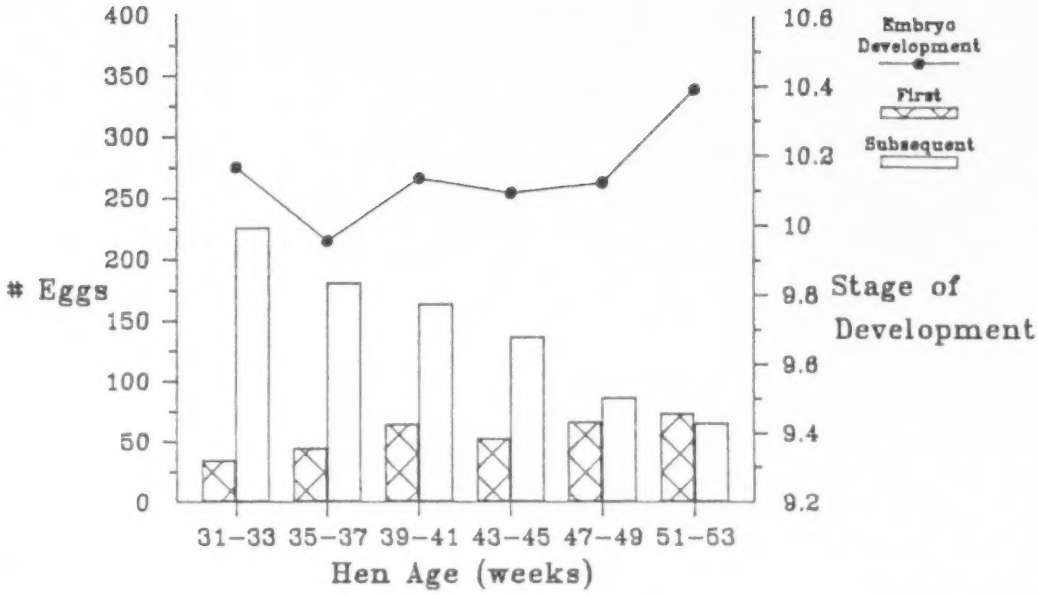


Figure V-5. Comparison of preincubation embryo development (Eyal-Giladi and Kochav (1976) - right y axis) with number of first and subsequent egg of a sequence (left y axis) and hen age (wk - x axis).

LITERATURE CITED

Eyal-Giladi, H., and S. Kochav, 1976. From cleavage to primitive streak formation: A complementary normal table and a new look at the first stages of the development of the chick. I. General Morphology. Dev. Biol. 49:321-337.

Hamburger, V., and H.L. Hamilton, 1951. A series of normal stages in the development of the chick embryo. J. Morph. 88:49-92.

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PUBLICATIONS

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Fasenko, G.M., F.E. Robinson, J.G. Armstrong, J.S. Church, R.T. Hardin, and J.N. Pettite, 1991. Variability in preincubation embryo development in domestic fowl. 1. Effects of nest holding time and method of egg storage. Poultry Sci. 70:1876-1881.

Fasenko, G.M., R.T. Hardin, F.E. Robinson, and J.L. Wilson, 1992. Relationship of hen age and egg sequence position with fertility, hatchability, viability, and preincubation embryonic development in broiler breeders. Poultry Sci. (Accepted for publication).

Published Abstracts:

Fasenko, G.M., F.E. Robinson, J.G. Armstrong, J.S. Church, R.T. Hardin, and J.N. Pettite, 1990. The effects of frequency of egg collection and method of storage on pre-incubation embryo development. Poultry Sci. 69(suppl. 1):49.

Fasenko, G.M., R.T. Hardin, F.E. Robinson, and J.L. Wilson, 1991. Factors influencing fertility and preincubation embryo development in broiler breeders. Poultry Sci. 70 (suppl. 1):41.